Symposia Biologica Hungarica 28

THE PINEAL GLAND

CURRENT STATE OF PINEAL RESEARCH

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Akadémiai Kiadó, Budapest

THE PINEAL GLAND
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Edited by

B. MESS, CS. RÚZSÁS, L. TIMA and P. PÉVET

(Symposia Biologica Hungarica 28)

This book contains the 24 invited lectures delivered at the Third Colloquium of the European Pineal Study Group. The papers, written by outstanding scientific authorities from all continents, cover the entire scope of the new trends that have emerged in pineal research in the last decade. The comparative morphology and physiology of the gland, the biochemistry of pineal hormones and their metabolism, the endocrine role with special reference to the reproduction in cyclic and in seasonal breeder species, as well as the role of environmental factors played in the regulation of the gland are all discussed in the book. A special chapter is devoted to the clinical application of the knowledge obtained in pineal physiology. Finally, two papers try to find the link between the data obtained mainly on laboratory rodents and in smaller part in human pathology by a thorough investigation of the pineal gland in primates.

This book is a useful tool for postgraduate students in need of a relatively short yet comprehensive survey on the pineal gland. Advanced scientists, morphologists, physiologists, endocrinologists, biochemists and comparative biologists will also find new data valuable for their future research in the field of pinealogy. Clinicians (internists, gynaecologists, psychiatrists) may also profit from the book in their diagnostic and therapeutic work.

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Proceedings of the Third Colloquium of the European Pineal Study Group, Pécs, Hungary, August 13–17, 1984.

Edited by

BÉLA MESS
CSILLA RÚZSÁS
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and
PAUL PÉVET

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PREFACE

The Third Colloquium of the European Pineal Study Group (EPSG) was held at the University Medical School Pécs, 13–16 August, 1984.

Subsequent to the successful past colloquia of our society (Amsterdam, 1978; Giessen, 1981) this was the first occasion where EPSG, a primarily European scientific association opened the doors for pinealogists from all over the world. It was a great privilege for Hungary, especially our University, to act as hosts to this meeting.

This volume follows the structure of the previous proceedings: only the papers of invited speakers are included. Unfortunately, the publication (or even the abbreviated version) of the 47 short oral communications and of the 46 poster presentations would have greatly exceeded the scope of this volume.

The morphology, physiology, biochemistry and endocrinology of the gland, as well as practical applications of these results, have been collated in this book, encompassing a wide range of the most significant latest trends in pineal research. It is hoped that this volume will help promote future research in this field.

Pécs, November 1984

Béla Mess
Csilla Rúzsás
Lajos Tima
Paul Pévet

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INTRODUCTORY SESSION

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

THE PAST SEVEN YEARS OF THE EUROPEAN PINEAL STUDY GROUP

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One may wonder whether a paper on the history of our EPSG is at all opportune after such a relatively short time of its existence. I can think of two reasons why I have been asked to deliver this speech. One is that I stood at its cradle, the other that, either consciously or unconsciously, seven is a symbolic or magic number in the mind of many. In Christianity, for instance, seven is one of the most frequently mentioned numbers throughout the Bible (1,2). According to the chapter Genesis God finished the creation of the world in six days taking some rest after this exacting job on the seventh day which is the reason why, according to the Mosaic laws, the last day of the week, a Saturday, is a day of rest and reflection (Lev. 23/3). In the seventh month after the big flood or deluge Noah's ark ran ashore on mount Ararat. The tale about the seven fat years which were followed by seven lean years is also very well known. We can only hope that this does not apply to the EPSG. In general, seven is the number of charity and grace. In the Bible reference is also made to the sevenfold gifts of the Holy Spirit, the seven deadly sins, and the seven joys and seven sorrows of Mary, the mother of Jesus. In the revelation of St. John the number seven frequently appears. However, not only in Christianity but also in many other religions, beliefs and fairy-tales the number seven plays an important role. Of interest from the viewpoint of a pinealogist is that, according to ancient Hindu belief, the human being has seven chakra's or centres of vital energy at his disposal. The supreme chakra, the sahasrara chakra, is located on the crown of the head. It was and by some is still held to be related to the pineal organ. In an earlier paper (3) the function ascribed to this chakra has been mentioned by me.

But let us now proceed to a different kind of reality, i.e. the history of the EPSG. It all started with the suggestion, made by Dr. Pévet to me in the autumn of 1976, to establish an association of scientists working in the field of pinealogy, a term which was only shaped later and which I prefer to the rather tongue-braking term pinealology. At first I was in doubt whether such a plan would have any sense, the number of scientific associations being already so very large, while, moreover, pineal research can be considered a special branch of

different disciplines of the natural sciences. I have also always had a dislike of scientific societies which can be suspected to have been founded due to the personal ambition of some people only cleverly distinguished by the most noble intentions. Considering , however, that cooperation and mutual support of workers in this rapidly expanding field could possibly be useful I agreed. In November 1976 we sent a letter to all European scientists, known to us to be working on or to have shown interest in the pineal organ, in order to ascertain whether they would support the foundation of a European Pineal Study Group explaining its purpose and advantages. We received many positive reactions and, in consequence, the foundation of the EPSG was officially announced and the election of a Council organized in a second letter, dated January 17, 1977. All pinealogists who had shown interest by returning their application form for membership were considered founder members and formed the electorate. In March 1977, Drs Collin, Ebels, Martini, Mess, Moszkowska, Nir, Oksche, Pévet, Vollrath and I were elected Council members out of 49 candidates by postal ballot, these Council members representing 6 European countries including Israel. In May of the same year this first Council nominated a Board in which Oksche acted ad vice-president, Pévet as secretary-treasurer and I as president.

The first meeting of the Council was held in Jerusalem on November 15, 1977, during an International Symposium on the Pineal Gland which was not organized by the EPSG. The Council approved of the statutes and by-laws for the EPSG which I had framed using those of the International Society of Developmental Biologists. According to democratic principles they were submitted for final adoption to all members of the Group at the first general assembly during the first colloquium of the EPSG, held in Amsterdam in November 1978. In January 1978, no less than 182 pinealogists from all over Europe were listed as active members. It is, perhaps, of historical interest to refer to some of the main articles of the statutes. It was stated that the EPSG is a scientific association aiming at the promotion of pineal research in its broadest sense, i.a. by periodically distributing a Newsletter, to be used as a channel of communication, and by organizing symposia and colloquia. Under symposia meetings were understood pertaining to a special field of pineal research to be organized by the Board on request of 20 active members and to be held at irregular intervals. To my knowledge such unidisciplinary symposia have never been realized although some small interdisciplinary meetings were locally organized. Furthermore, the statutes state that every three years international colloquia pertaining to all fields of pineal research shall be organized by the Board and the chairman of the local committee. A general assembly of the members will have to be held once every three years during such a colloquium. This assembly shall, on recommendation by the then sitting Board, elect a new Board from the members of a new Council, some months before nominated by postal ballot by all members. Furthermore, the statutes explicitly stipulate that the EPSG will endeavour to cooperate with any similar group founded outside Europe. In a number of articles the different categories of members — active, emeritus, supporting and honorary members —

were enumerated and their rights defined.

The first colloquium was held in Amsterdam from November 20 to 24 in 1978. It gave the 95 participants a good opportunity to learn what was going on in pineal research in Europe. 18 Major lectures and 44 short communications were delivered, their scientific level being very satisfactory. Much time was also devoted to discussion. The general assembly held during this colloquium approved of the statutes and by-laws elaborated by the provisory Council in 1977, while this Council was also officially nominated. At the colloquium 18 European countries were represented including Israel.

Dr. Oksche excellently summarized the results in a more general way. He stressed the importance of a comparative approach to pineal problems and of the modern technical tools now available to solve them, especially as pineal biochemistry and pharmacology are concerned. He also pointed to the need of chemical identification of specific pineal peptides and other pineal compounds in relation to pineal function and their control of hypothalamic activity specially regarding gonadotropic and antigonadotropic mechanisms. Dr. Oksche also rightly stressed the fact that, although it is now well-established that the pineal is a component of the neuroendocrine system, next to being a link in nervous messages, the organ is of a complexity not easy to unravel. He also mentioned that the vast number of functions attributed to the pineal makes one feel uneasy and he was of the opinion that the final break-through is still ahead of us. In my opinion this dilemma still holds because, although our knowledge about pineal structure and biochemistry has considerably increased since 1978, the exact function of the organ in relation to other endocrine structures is scarcely better known.

The proceedings of this colloquium were published in a volume of the series "Progress in Brain Research" in 1979, Dr. Pévet and I acting as editors (4).

The second colloquium, extremely well-organized by Dr. Oksche and his staff, was held three years later in Giessen from July 1 to 4, 1981. It was attended by 101 participants. The programme covered a wide field of pineal research and consisted of 20 major papers and short communications, finally published. For the first time, opportunity was given to exhibit posters, while, again, ample time was devoted to discussions. The results were summarized by Dr. Mess. He mentioned that the presentations had given much new information on pineal structure and function, including the phylogenetic aspects of pineal morphology and physiology, and the neuroendocrine relationships between the pineal and other neuroendocrine systems, especially concerning its role in the biochronometrical regulation of reproductive functions. The new biochemical data offered to raise the hope that the isolation and purification of new active principles can be expected in the near future. He also stressed the fact that clinical and pathological investigations prove that pineal research is also of importance for clinical work and therapy while regretting that, due to unforeseen circumstances, no presentation could be devoted to the pineal of subhuman primates. In the opinion of Dr. Mess also, the crucial problems of pineal physiology still remain open to discussion. He was, however, optimistic that the car-

dinal questions in this field will be solved stepwise. The proceedings of this colloquium were already published in the same year in the series "Developments in Endocrinology", Drs Oksche and Pévet acting as editors (5).

At the start of the Giessen colloquium a Council meeting was held. The undersigned, acting as president, welcomed the newly elected Council members Drs Arendt, Karasek and Trentini. Then he gave the chair to Dr. Oksche, the newly elected president. Dr. Mess was invited to act as vice-president while Dr. Pévet was asked to continue his work as secretary-treasurer. Furthermore, the Council decided to propose to the general assembly the nomination of Drs J.Axelrod, A.Moszkowska, S.Milcu, B.Scharrer and L.Thiéblot as honorary members of the EPSG in gratitude of their important contributions to pinealogy, and the nomination of the undersigned as honorary president who accepted this honour most gratefully. Dr. Mess kindly agreed to organize, in collaboration with the Board, the third colloquium, scheduled for 1984, in Pécs.

Some modifications of the statutes as prepared by the undersigned were approved of by the Council which decided to submit them to the general assembly for final approval. The most important modification was that, instead of "active members", the terms "ordinary members" and "associate members" were introduced. According to the revised statutes European pinealogists can apply for ordinary membership, while non-Europeans obtain the right to join the EPSG as associate members. The reason for this revision, which is not quite without historical interest, was the following. At the end of 1980 an Australian Pineal Study Group was organized by Drs Kenny and Armstrong. Its first major activity was the arrangement of a symposium on pineal structure and function in May 1981. This symposium was sponsored by the Anatomical Society of Australia and New Zealand and held during a meeting of that Society at the Anatomy Department of the University of Melbourne. Members of this Australian group also participated with the Australian Broadcasting Commission in the production of a documentary on the pineal organ entitled "The Third Eye", which was shown on Australian television. However, what was possible in Australia was evidently not so in the United States due to reasons we could only guess. Dr. Pévet and I received some letters from American colleagues reproaching the EPSG of being an exclusive club for Europeans only. In answer we asked our friends in the States to found their own Pineal Study Group, mentioning that we wanted to keep the EPSG European at least during some years to await how matters would develop within this Group. We also expressed our joy that American pinealogists were evidently interested in the activities of the EPSG, while, because non-Americans can apply for membership of the American Association of Anatomists which exists next to a European Anatomical Association, and because we did not want to be quite so exclusive we proposed to introduce an associate membership, also in view of the members of the Australian Pineal Study Group. We, however, stipulated slight restrictions regarding the right of voting of such members. The future will learn whether a true International Association of Pinealogists can possibly be founded. After some discussion, the revised statutes were accepted by the general

assembly. Since, a number of distinguished American colleagues joined the EPSG and we are most glad to have some of them among us here. — The assembly, furthermore, agreed to the nomination of the honarary members and of the honorary president who received a commemoration medal.

In the Newsletter of March 1984, 229 honorary and ordinary members and 40 associate members of the EPSG are listed, while, in the mean time, these numbers did even slightly increase.

Just a few words should be said about our Newsletter which appears two times a year. From the first issue on, which appeared in January 1878, Dr.Pévet, acting as its editor, devoted much time to this job. First he was assisted by Dr. E.Tapp, Great Britain, and later by Dr. J.Arendt, acting as co-editors, while Miss J.Sels, secretary at the Netherlands Institute for Brain Research, has given excellent secretarial assistance during all of the past seven years. The Newsletter forms a most important link between the members of the EPSG by dealing with all activities of our association including the announcements and programmes of the colloquia, the minutes of the Council meetings and of the general assemblies. Regularly, a list of members with their addresses is published. In a chapter called "Pineal News" new books on the pineal and relevant congresses and symposia, not organized by the EPSG, are announced while reviews of such activities and of theses are also given. Moreover, facilities for pineal research in different laboratories are mentioned. The interesting series "Ancestors and Pioneers in Pinealogy" is not only of much historical importance, it makes reading of the Newsletter very worthwhile. The "Letter of the President", published in every issue, deals with several questions related to our association or to scientific research in general. Once more it should be pointed out that the Newsletter should not only be passively read by the members, but that these should also actively contribute to its contents by giving all sorts of information useful for pineal research and our association.

In concluding it would seem to me that the past seven years did prove that the foundation of the European Pineal Study Group in 1977 was well warranted. Our Group has developed in a satisfactory way and its general importance for the members is evident. The EPSG did also much to convince the scientific community that pineal research is not only of interest to some non-clinical hobbyists, but also of practical importance for clinical work and therapeutics. We do not know what the future keeps in store. It can, however, be expected that, due to the world-wide economical recession, it will become increasingly difficult for young scientists to find adequate jobs. Moreover, the development of basic research will possibly be hampered by more demands for practical applicable scientific work. Therefore, cooperation and mutual support will be of increasing importance. During the next years the Board and the Council will, I am sure, have a more difficult task than during the past years. It is necessary that the Board, with the help of the entire Council, will give good leadership being alert in regard of the impact of the present socio-economical developments on the scientific world in general. However, much will also depend on the right spirit of all of the members of our

EPSG. The value of any association like ours is not exclusively dependent on the leadership of its Board, but also on the quality, scientifically as well as personally, of its individual members. In the present world of politics it appears to be most difficult to reach general international agreements and unity, i.a. due to the fact that the issues at stake and the history and traditions of the different countries and their economical problems are so different. For us, members of an international scientific organization who have the rather simple and restricted aim to perform and promote pineal research, it is certainly more easy to obtain and keep a fruitful cooperation. It is, however, necessary that all members are aware of their personal responsability and willing to support our association actively. May the future of the European Pineal Study Group be a prosperous one !

References

1. LIPFFERT, K. : Symbol-Fibel, eine Hilfe zum Betrachten und Deuten Mittelalterlicher Bildwerke, Stauda Verlag, Kassel, 1961.
2. FERGUSON, G. : Signs and Symbols in Christian Art, Oxford University Press, London-Oxford-New York, 1972.
3. ARIËNS KAPPERS, J. : Evolution of pineal concepts. In: The Pineal Organ: Photobiology — Biochronometry — Endocrinology, A.Oksche and P.Pévet, eds. Developments in Endocrinology, *14*, Elsevier/North-Holland Biomedical Press, Amsterdam - New York - Oxford, 1981, pp. 3-23.
4. The Pineal Gland of Vertebrates including Man, J.Ariëns Kappers and P.Pévet, eds. Progress in Brain Research, *52*, Elsevier/North-Holland Biomedical Press, Amsterdam - New York, 1979.
5. The Pineal Organ: Photobiology — Biochronometry — Endocrinology, A.Oksche and P.Pévet, eds. Developments in Endocrinology, *14*, Elsevier/North-Holland Biomedical Press, Amsterdam - New York - Oxford, 1981.

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

PRESIDENT'S ADDRESS
COMPARATIVE ASPECTS IN PINEAL RESEARCH

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At the Second Colloquium of the European Pineal Study Group (Giessen 1981) particular emphasis was placed on comparative aspects of pineal research (cf. Oksche and Pévet 1981). In this context, the following concepts were postulated:

1) The pineal organ is a derivative and integral component of the brain.
2) In evolutionary terms, it can be considered as an extraretinal photoreceptor, biological clock, and endocrine gland.
3) In phylogeny, the pineal organ has undergone profound changes leading to remarkable variations in its sensory and secretory apparatus.

My present reflections are based on more than two decades of personal comparative research (cf. Oksche 1983 a, b). Nearly a century after the discovery that the eye-like parietal bodies of lower vertebrates are homologues of the pineal organ of mammals (for references, see Studnička 1905, Bargmann 1943), Dodt and associates succeeded in demonstrating the direct sensitivity of these organs to light (cf. Dodt 1973). My joint work with Dodt and his colleagues (cf. Oksche 1971, 1983 a, b, 1984) was paralleled by the extensive comparative studies of Collin (cf. Collin 1971, Collin and Oksche 1981). Independently, we came to the conclusion that the secretory parenchymal elements of the pineal gland - the pinealocytes - are derivatives of primary sensory cells (Fig. 1). Moreover, from our observations I suggested that in pineal photoreceptor cells the photic information can be translated into a neuroendocrine response. In consequence, this led me to the assumption that sensory pinealocytes may be regarded as photoneuroendocrine cells of neuronal origin (Oksche 1970, 1971). A number of recent reports (e.g. Ekström 1983, Falcon 1984) has shown that this working hypothesis is still of current interest.

It is not by mere accident that a comparative synopsis based on our results (Fig. 1; see Oksche 1983 a) adds pertinent ultrastructural data to the classical anatomical diagrams of Studnička (1905). Looking back at the European contribution to the investigation of the pineal organ it is striking that this contribution has always had a very distinct evolutionary and comparative accent. Outstanding representatives of this conceptual line were F. K. Studnička and W. Bargmann, authors of treatises with eminent and long-lasting influence on pineal research (Studnička 1905, Bargmann 1943). My following comments will pay tribute to these great masters who have one characteristic feature in common: Both of them

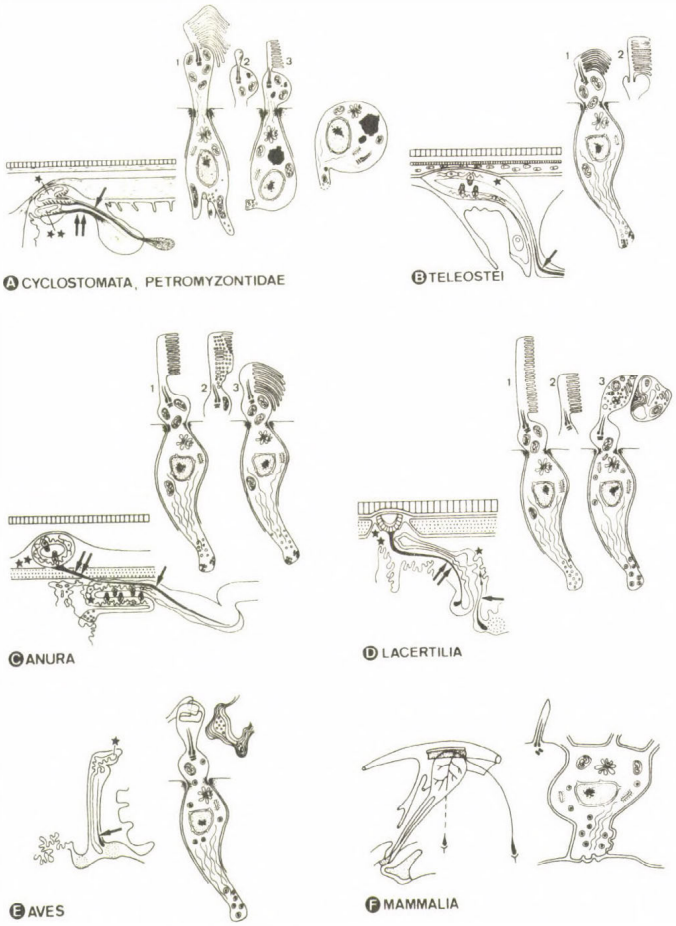

Fig. 1. Comparative representation of parietal eye - pineal complexes. Diagrammatic midsagittal views in relation to the respective features of the pinealocytes of the receptor type. Single star: pineal organ (epiphysis cerebri); double star: parapineal organ (cyclostomes, lacertilians) or frontal organ (anurans); single arrow: pineal tract: double arrow: parietal-eye (lacertilians) or frontal-organ tract (anurans) Note diversity in ultrastructural details of the pinealocytes (1-3) indicating changes in outer segment structure and secretory capacity. From Oksche (1983a); courtesy Plenum Publishing Corporation.

were well-trained, open-minded cytologists and neuroanatomists with a surprisingly wide spectrum of research interests.

When, in 1905, Studnička (at an age of 35) published his monograph on parietal organs in Oppel's"Lehrbuch der Vergleichenden Mikroskopischen Anatomie der Wirbeltiere", he was already well known as the author of a comprehensive treatise on ependyma and ependymal organs (Studnička 1900). This was a real conceptual breakthrough in the analysis of the general morphologic pattern of the brain. Studnička's ependymal gliocytes are identical with the "tanycytes" of today, and his "ependymal organs" represent a major component of the "circumventricular organs". In my opinion this avantgardistic and largely epochal contribution overshadows his later systematic efforts to elucidate the nature of exoplasmic substances and structures. The lasting influence of Studnička's monograph on the complex problem of parietal organs (derivatives of pineal and parapineal primordia) depends on the precise and critical overview given; its value is not diminished by a somewhat cumbersome style. Subsequent to discovery of the frontal organ ("pineal eye") by Stieda in 1865 (originally misinterpreted as a gland), a comprehensive body of comparative descriptions of similar organs, partly identified as homologues of the mammalian pineal organ, accumulated until the end of the nineteenth century.

Studnička's treatise was the first comprehensive synopsis in this field. His analysis of the microscopic structure of parietal organs suffers only partly from the limited resolving power of the light microscope. Concerning the parietal organs, Studnička came to the following conclusions: "These organs served originally, as one may assume with a certain degree of certainty, photoreceptive functions, however, they persisted in this form only in relatively few groups of vertebrates". "One of the parietal organs, the pineal organ, has undergone a strange change in function; a sense organ was transformed into a gland of complex structure and enigmatic function. This functional alteration was accompanied by changes in the structural pattern of the organ; however, the onset of these changes is difficult to recognize, namely the structure of a functioning sensory pineal organ may also alter in a manner that it, at least partly, resembles a gland" . *)

A further landmark was made when Bargmann, in 1943 (still in the era of light microscopy), published his brillantly written and illustrated monograph on the epiphysis cerebri (pineal organ). Bargmann (then at the age of 37) had already published a considerable number of important papers on different topics, including the fine structure of the kidney, lymphoepithelial organs (thymus) and endocrine glands (thyroid). He reached the pinnacle of his scientific career only a decade later by his fundamental discoveries in the field of neurosecretion, followed by outstanding electron-microscopic analyses of various tissues and organs. Bargmann's monograph, a contribution within Möllendorffs "Handbuch der Mikroskopischen Anatomie des Menschen", is a document of his extraordinary engagement and great skill as a comparative anatomist. The concluding paragraph in the epilogue of this monograph, largely based on comparative evidence, contains scientific statements of eminent importance and highly predictive character:

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*) Translated by A. O.

"The specific cellular elements of the pineal organ of the lower vertebrates display the character of sensory cells. Their morphological features allow the assumption that they are capable of secretory functions. One may tend to attribute to these elements, which both genetically and functionally resemble neurons, a synthetic capacity, last but not least with reference to the secretory (neurocrine) activity of nerve cells, particularly characteristic of the diencephalon. One may assume that in the surface-exposed pineal organs of lower forms the secretory activity of pineal receptors depends directly on illumination. The transformation of the pineal organ into a compact, highly vascularized body is accompanied also by a change in the structure of pinealocytes; after regression of their receptive segments the sensory cells are transformed into pinealocytes of the mammalian type. This results in a loss of the polar structure of the cells. It is an experimental task to investigate whether pinealocytes, after prevention of direct exposure to light, are still capable of receiving impulses via neural pathways coupled to the optic system thereby serving synthetic functions. Thus, the comparative approach leads to a working hypothesis that will most likely be fruitful in understanding the pineal organ". *)

As already outlined on the occasion of the First Colloquium of the European Pineal Study Group (Amsterdam 1978; see Oksche and Hartwig 1979), the comparative concept also proved to be the most inspiring in the functional studies of Karl von Frisch (1911) and Ernst Scharrer (1928). From their experiments both investigators concluded that the pineal organ of teleosts may be regarded as a principal, however, not the sole site of extraocular photoreception. From our present point of view, this epochal pioneer work provided a keen and still valid concept of photoneuroendocrine systems; it helped to cast light on basic functional properties of the pineal organ.

These examples clearly show that in pineal research the comparative approach is not only of heuristic value. It establishes the basis for future ultrastructural, immuncytochemical and biochemical investigations extending as far as to the molecular level. As reviewed by Vollrath (1981), comparative aspects have become increasingly important also for the interpretation of structures and functions of the mammalian pineal organ. They help to understand species-dependent differences, individual variations and topographical specializations.

The pineal complexes of submammalian vertebrates, including their specific types of sensory cells, the pattern of their intrinsic circuitries and their reciprocal neural connections, provide a key for the interpretation of the pineal gland of mammals. It is not the objective of the present remarks to list and evaluate recent progress in these areas. Several of the plenary lectures of the present conference are focussed on these topics. Therefore, only a few considerations will be made.

The functionally very important question of the plurality of pineal sensory cells is still open to discussion. This problem involves, on the one hand, the apparent dualism of sensory and sensosecretory elements (Meiniel 1981, Falcon 1984), on the other hand, the occurrence of different photopigments in photoreceptor cells morphologically displaying only cone-type outer

*) Translated by A. O.

segments (Hartwig 1975, Vigh-Teichmann and Vigh 1983). In this area new comparative investigations with improved methods are in progress (see also Ekström 1973). They may lead to a better understanding or even clarification of the achromatic and chromatic types of response of sensory pineal organs (cf. Dodt 1973).

The intrinsic circuitry of pineal sense organs is another topic deserving thorough comparative investigation. In addition to the problem of photopigments, these circuitries are of basic importance with respect to the archromatic and chromatic reactions observed in photoreceptive pineal organs. Complementary to further efforts employing different tracers, the identification of the neurotransmitters of the individual neuronal elements is one of the major aims of future structural and functional analyses. In the mammalian pineal gland increasing attention should be paid to the nexus-type connections displayed by individual and clustered pinealocytes (cf. Vollrath 1981). It is possible that precursors of these structural differentiations can be found in sauropsid species.

Finally the reciprocal neural connections (pinealofugal and pinealoptal pathways) are an issue of great comparative interest. The message delivered by sensory pineal organs to the brain has still not been deciphered. For demonstration of the neural pathways the use of modern tracer methods has been very useful (for references, see van Veen 1981; Ekström 1983, Korf and Møller 1983). These studies have not only supplied us with a more precise knowledge of the central projection sites of sensory pineal organs but also with the insight that the pineal organ of mammals receives, in addition to the sympathetic input, a conspicuous innervation originating in the central nervous system (cf. Quay 1984). Again, the pineal pathways in sauropsids deserve particular comparative attention. Very obviously, the central pinealopetal projections of mammals had their forerunners in submammalian vertebrates.

The character of the avian pineal organ should be reconsidered in the light of new structural and functional findings. Due to the irregular geometry and the apparently poor differentiation of the outer segments of its receptor-like cells (cf. Collin and Oksche 1981), and the lack of a typical electrophysiological response to illumination (cf. Dodt 1973), they were thought to be rudiments not capable of direct photic functions (cf. Oksche 1971, Dodt 1973). However, recent investigations of Deguchi (1982) indicate the existence of a rhodopsin-like photoreceptor in the cell membrane of chicken pinealocytes; in organ culture this photoreceptor controls the circadian rhythm of the activity of serotonin N-acetyltransferase. The chicken pineal is clearly photoreceptive; this is also corroborated by Takahashi (1982) who investigated light-dependent melatonin rhythms in tissue culture. In addition, by means of electrophysiological recording, Semm and Demaine (1983) observed electrical responses to direct photic stimulation in the pineal organ of the pigeon. Finally, in the pigeon, Vigh-Teichmann and Vigh (1983) were able to show a positive immunoreaction against bovine rhodopsin in the lamellar outer-segment structure of pinealocytes. For comparative reasons more attention should also be paid to the situation in reptiles, along the guidelines presented by Quay (1979). On the other hand, the possibility of some kind of direct response of mammalian pinealocytes to light is a matter of discussion; this capacity may serve the translation of optical signals into chemical responses.

It is hardly possible to understand the pineal organ of amniotes without knowledge of its evolutionary past (cf. Oksche 1983b). Phylogenetic analyses of central nervous structures, however, depend basically on the classic rules of anatomical comparison (Kuhlenbeck 1967). First of all, it is crucial to avoid confusing morphologic homology with analogy. Further, concepts of similarities indicating anatomical equivalents do not replace the notion of homology in its strict original sense. According to Kuhlenbeck (1967) only sets of formed elements that fit by topologic transformation into a component of the general morphological pattern can be regarded as homologues.Within this conceptual framework recent histochemical, immunocytochemical and quantitative methods can be widely used, thus furnishing the comparative analysis with new tools and leading to insights more closely correlated to functional aspects. It should, however, be noted that today also immunochemists speak of "homologies", when, e.g., in peptides homologous sequences of amino acids have been shown by radioimmunoassay. This approach may also help to interpret the similarities and differences of opsins occurring in retinal and extraretinal (pineal) photoreceptor cells (cf. Vigh-Teichmann and Vigh 1983). Clear-cut definitions are needed to avoid confusion when research is in such a state of flux as at the present time.

The principles of comparative anatomy, applied on an ontogenetic basis and focussed mainly on the pattern of neural connections, are of particular value when attempting to identify derivatives of pineal or parapineal primordia. However, even a precise knowledge of these facts will not solve the problem of the functions of the individual organs; for the latter purpose, an analytic and experimental approach is obligatory. Thus, microspectrophotometric studies (Hartwig 1975; see also Oksche and Hartwig 1979) have shown that different (rod- and cone-like) photopigments may occur in outer segments of pineal sensory cells displaying a rather uniform cone-like arrangement of their lamellar systems. This is accord with observations on the retina of the lateral eyes where rhodopsin can be found in cone-like photoreceptor cells, and vice versa (Dodt 1962). Furthermore, due attention must be paid to geographical, ecological and environmental factors (cf. Ralph 1975) having influence on endogenous rhythms.

With satisfaction I realize the increasing efforts of the members of our group to establish interdisciplinary research and to overcome the traditional methological barriers between the morphological and functional disciplines. In conclusion, however, I strongly re-emphasize my favorite idea that pineal research - for the sake of its quality - should never be separated from the mainstreams in neurobiology and endocrinology.

SUMMARY

The present analysis is based on more than two decades of personal comparative work and intense interdisciplinary research cooperation with other groups. The pineal organ is an integral component of the brain. In evolutionary terms, it can be considered as an extraretinal photoreceptor, biological clock and endocrine gland. The diversity of structure and function of the pineal complex reflects a high degree of evolutionary and adaptive capacity. In phylogeny, the pineal organ has undergone profound changes, which have produced remarkable variations in its sensory and secretory apparatus. It is, however, of crucial importance that the secretory parenchymal elements of the pineal organ - the pinealocytes - are derivatives of primary sensory cells displaying properties of photoneuroendocrine neurons. The pineal complexes of submammalian vertebrates, including the pattern of their intrinsic circuitry and their reciprocal neural connections, provide an important key for the interpretation of the pineal gland of mammals. Attention must be paid to new methodological approaches when establishing homologies or attempting to define functional equivalents. In pineal research the comparative approach is valuable not only for heuristic purposes. It establishes the basis 1) for future ultrastructural, immunocytochemical and biochemical investigations extending as far as to the molecular level, and 2) for further elucidation of pineal functions.

This contribution is dedicated with great personal appreciation to Professor Heinz Rollhäuser of Münster, my predecessor in Giessen, on the occasion of his 65th birthday.

REFERENCES

Bargmann W (1943) Die Epiphysis cerebri. In: von Möllendorf W (ed) Handbuch der mikroskopischen Anatomie des Menschen. Vol IV/4, Springer Berlin, pp 309 - 502

Collin JP (1971) Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In: Wolstenholme GEW, Knight J (eds) The pineal gland. Churchill Livingstone London, pp 79 - 125

Collin JP, Oksche A (1981) Structural and functional relationships in the nonmammlian pineal gland. In: Reiter RJ (ed) The pineal gland, Vol I: Anatomy and biochemistry. CRC Press Boca Raton, pp 27 - 67

Deguchi T (1982) Endogenous oscillator and photoreceptor for serotonin N-acetyltransferase rhythm in chicken pineal gland. In: Aschoff J, Daan S, Groos G (eds) Vertebrate circadian systems. Springer Berlin Heidelberg, pp 164 - 172

Dodt E (1962) Über die Grundvoraussetzungen der Duplizitätslehre des Sehens. Naturwissenschaften 49: 530 - 533

Dodt E (1973) The parietal eye (pineal and parietal organs)of lower vertebrates. In: Jung R (ed) Handbook of sensory physiology, Vol VII. Springer Berlin, pp 113 - 140

Ekström P (1983) Photoneuroendocrine systems in a teleost fish, the three-spined stickleback, Gasterosteus aceleatus L. Thesis, University of Lund, Lund

Falcon J (1984) Identification et propriétés des cellules photoneuro-endocrines de l'organe pinéal. Thèse, Université de Poitiers, Poitiers

Frisch K v (1911) Beiträge zur Physiologie der Pigmentszellen in der Fischhaut. Pflüger's Arch ges Physiol, pp 138 - 139

Hartwig H G (1975) Neurobiologische Studien am photoneuroendokrinen System. Habilitationsschrift (Thesis), Justus-Liebig-Universität, Giessen

Korf HW, Møller M (1984) The innervation of the mammalian pineal gland with special reference to central pinealopetal projections. Pineal Research Reviews 2: 41 - 86

Kuhlenbeck H (1967) The central nervous system of vertebrates, Vol I. Kargel Basel

Meiniel A (1981) New aspects of the phylogenetic evolution of sensory cell lines in the vertebrate pineal complex. In: Oksche A, Pévet P (eds) The pineal organ: photobiology-biochronometry-endocrinology. Elsevier/North-Holland Biomedical Press Amsterdam, pp 27 - 47

Oksche A (1970) Zur Differenzierung sensorischer und sekretorischer Strukturelemente im Zentralnervensystem. Verh Dtsch Zool Ges 64: 72 - 79

Oksche A (1971) Sensory and glandular elements of the pineal organ. In: Wolstenholme GEW, Knight J (eds) The pineal gland. Churchill Livingstone London, pp 127 -146

Oksche A (1983a) Aspects of evolution of the pineal organ. In:Axelrod J, Fraschini F, Velo GP (eds) The pineal gland and its endocrine role. Plenum Press New York, pp 15 - 35

Oksche A (1983b) Comparative research on the pineal organ: personal reflections. In: Pévet P, Arendt J (eds) EPSG Newsletter. EPSG Amsterdam, pp 11 - 20

Oksche A (1984) Evolution of the pineal complex: correlation of structure and function. Ophthalmic Res 16: 88 - 95

Oksche A, Hartwig HW (1979) Pineal sense organs - components of photo-neuroendocrine systems. In: Kappers JA, Pévet P (eds). The pineal gland of vertebrates including man. Progress in brain research, Vol 52. Elsevier/North-Holland Biomedical Press Amsterdam, pp 113 - 130

Oksche A,Pévet P (1981) (eds) The pineal organ. Photobiology - biochronometry-endocrinology (Developments in endocrinology, Vol 14). Elsevier/North-Holland Biomedical Press, Amsterdam New York Oxford

Quay WB (1979) The parietal eye - pineal complex. In: Gans C, Northcutt RG, Ulinski P (eds) Biology of the reptilia, Vol 9 (5). Academic Press London New York, pp 245 - 406

Quay WB (1984) Pineal function in mammals: a reconsideration. Pineal Research Reviews 2: 87 - 112

Ralph CL (1975) The pineal organ and geographical distribution of animals. Int J Biometeorol 19: 289

Scharrer E (1928) Die Lichtempfindlichkeit blinder Elritzen (Untersuchungen über das Zwischenhirn der Fische I.) Z Vgl Physiol 7: 1

Semm P, Demaine C (1983) Electrical responses to direct and indirect photic stimulation of the pineal gland in the pigeon. J Neural Transmission 58/ 281 - 289

Stieda L (1865) Über den Bau der Haut des Frosches (Rana temporaria). Arch Anat Physiol 1865: 52 - 79

Studnička FK (1905) Die Parietalorgane. In Opel A (ed) Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere, Teil 5. Fischer Jena, pp 1 - 254

Takahashi JS (1982) Circadian rhythms of the isolated chicken pineal in vitro. In: Aschoff J, Daan S, Groos G (eds) Vertebrate circadian systems. Springer Berlin Heidelberg, pp 158 - 163

Van Veen Th (1981) A study on the basis for Zeitgeber entrainment. With special reference to extraretinal photoreception in the eel. Thesis, University of Lund, Lund

Vigh-Teichmann I, Vigh B (1983) The system of cerebrospinal fluid-contacting neurons. Arch Histol jap 46: 427 - 468

Vollrath L (1981) The pineal organ. In: Handbuch der Mikroskopischen Anatomie des Menschen, Vol. VI/7, Oksche A, Vollrath L (eds) Springer Berlin Heidelberg New York.

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

NEV TRENDS IN PINEAL RESEARCH

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This year represents the thirtieth anniversary of the publication of the book by Kitay and Altschule / 1954 / on the pineal gland that was later quoted by Wurtman / 1982 / as a "landmark monograph". This singular citation should not in any way minimize the pioneering research of so many other investigators, oftly surveyed in the several issues of the EPSG Newsletter. Like mushrooms in the forest after a warm, fertilizing spring rain, publications dealing with the varied aspects of pineal structure and function emerged in huge number in subsequent years. It is not my present intent to survey the history of "pinealogy"; rather - as promised in the title - to outline the latest trends experienced in pineal research of recent years. Therefore, I shall skip 20-25 years, and since the perspectives and future of this branch of science will be discussed by dr. Reiter at the closing session of our Colloquium, only those new aspects briefly will be summarized, hopefully reflecting our present program.

It is an extremely rare occurrence in the history of science that an entirely new idea or discovery would revolutionize one or an other branch of science, e.g. the detection of X-rays, radioactivity, or development of radioimmunoassay techniques in endocrinology. Permit me to be very sincere and critical. Perhaps, I can afford to say that such a type of "revolutionary" event hardly can be found in the pineal research in our decade. However, a spectacular number of noticeable trends, methods and observations have underscored the progress in pineal research, and a rapidly increasing number of investigations became involved in this field of science.

The most important new trends, discussed here, are the followings:

1. Comparative biology, morphology and physiology of the pineal gland from the lower vertebrates through primates up to man.
2. Isolation and physiological function of different hormones or hormone-like principles of the pineal.
3. Pineal and adaptation. The role of environmental factors, other than light, in the regulation of pineal function.
4. The relationships of the pineal gland with the general neuroendocrine circuits. Reciprocal effects of pineal hormones and the central nervous system.
5. Pineal research in clinical practice. The use of pineal hormones in diagnostics and therapy.

Professor Oksche has brilliantly summarized the comparative aspects of pineal research. Comparative biologists have learned much from pinealogists and vice versa. The thorough analysis of the comparative morphology and physiology of the pineal gland, starting with the lower vertebrates, and progressing through the birds, marsupials, and ordinary laboratory rodents culminating with the subhuman primate and the human, surely will help to approach a series of problems in understanding the function of this organ. This important aspect hopefully will be sufficiently reflected by the program of our present Colloquium.

From the very beginning, two groups of bioactive compounds, melatonin /Lerner et al. 1959/ and proteins /Moszkowska, 1965/, represented the two most reasonable candidates for "pineal hormones". In the last decade / Table 1 /, great efforts were made to clarify the structure and function of the different hormones, or hormone-like principles of this gland. A series of new compounds, e.g. arginine vasopressin /AVP/ /Pavel, 1978/; different methoxyindole-amines /Pévet et al. 1981; Balemans et al. 1983/; and hydroxyindole-amines /Young and Anderson,1982/ pteridines /Ebels, 1981/; threonyl-seryl-lysine /TSR; Orts et al. 1980/ equally were proposed to be synthesized by the pineal, and to play important roles in the physiological actions of the organ. Others, such as Reiter and Vaughan /1977/, and Benson /1977/, tried to couple the role of the two main groups of hormones, proposing that melatonin

/or indoles/ influence the secretion of the biologically active protein hormones of the pineal. This crucial question is far from being solved. The program of our Colloquium devotes an entire day to this complex question.

Table 1.

HORMONES OR HORMONE-LIKE BIOACTIVE PRINCIPLES DETECTED IN THE PINEAL GLAND

Hormones	References
Melatonin	Lerner et al. 1959
Peptide-fractions /F_2 - F_3/	Moszkowska, 1965
Arginine-vasotocin /AVT/	Pavel, 1978
Methoxyindoles	Pévet et al. 1981; Balemans et al. 1983
Hydroxyindole-amines	Young and Anderson, 1982
Pteridines	Cremer-Bartels, 1979 Ebels, 1981
Threonyl-seryl-lysine	Orts et al. 1980

Besides the classical light-induced daily rhythm, and the endogenous circadian rhythm of melatonin and serotonin secretion /Axelrod and Wurtman, 1966/, a series of other environmental factors were investigated: olfactory impulses by Vaughan et al. /1978/; thermal impulses by Vivien-Roels /1981/; feeding, moisture etc. by Hoffman/1982/. These factors were proposed to play integrative roles in the function of the pineal gland acting as neuroendocrine transducers in the environmental adaptation of the higher vertebrates. The discussion of this new and highly interesting line is the main purpose of one of the sessions in the program of our Colloquium.

I would like to turn now to my favorite problem. A few years ago, my strongest opposition to the mode of approach of pineal endocrinology, discussed in the last Colloquium of the EPSG, was the tendency of "isolation" of the pineal from the general neuroendocrine system. Many investigations dealt with different problems such as "pineal and reproduction", "pineal and adrenals", "pineal and environment" or even

"pineal and sleep", etc., as if the pineal were an independent "master" gland of the endocrine system. The last decade brought important change and perceptions to this aspect. Increasingly, perhaps the majority of the papers today consider the pineal gland to be an important modulator or switch between or parallel to the central nervous system and the pituitary-target organ circuits. In this manner, pinealogists can now communicated with general neuroendocrinologists, that is, at least in my opinion, an important development. Therefore, pineal research is no longer regarded with scepticism by the neuroendocrinologist, as was the case 20-30 years ago /Reiter, 1982/. This trend was strictly coupled with the development of a new line investigating the effects of the pineal gland and its hormones exerted on different sites and functions of the central nervous system. A separate session at this present meeting deal with the different aspects of this promising but highly complicated problem.

Finally, one of the most important advances in pineal research was the adaptation to the requirements of practical life. For example, animal husbandry technology has already applied different programs of artificial lighting conditions with success. However, since this Colloquium has been organized within the walls of a medical university, the main emphasis was placed on its use in clinical practice. The development of different RIA systems suitable for the determination of pineal hormones in the blood, cerebrospinal fluid and urine /Arendt et al. 1977; Wetterberg et al. 1978/ had an important role in the progress of pineal research. To underscore the importance of pineal research performed in human patients, and its application in clinical diagnosis and therapy, the last whole-day session, closing our Colloquium, will be devoted to this category.

Such are the main and most promising new trends, characteristic of our present-day pineal research. Hopefully, all these problems will be sufficiently discussed here, and our Colloquium will add new data and dimensions to the further progress of pineal research.

REFERENCES

Arendt J., Wetterberg L., Heyden T., Sizonenko P.C., Paunier L. /1977/ Radioimmunoassay of melatonin: human serum and cerebrospinal fluid. Hormone Res. 8: 65-75

Axelrod J., Wurtman R.J. /1966/: The pineal gland: a biological clock. in: Problems actuels d'endocrinologie et de nutricion. Symp. internationale sur la neuro-endocrinologie. Série No 10: 201-212

Balemans M.G.M., Mans D., Smith I., Van Benthem J. /1983/: The influence of GABA on the synthesis of N-acetylserotonin, melatonin, O-acetyl-5-hydroxytryptophol in the pineal gland of male Wistar rat. Reprod. Nutr. Develop. 23: 151-160

Benson B. /1977/: Current status of pineal peptides. Neuroendocrinology 24: 241-258

Cremer-Bartels G. /1979/: The effect of pteridines on multiple forms of Hydroxyindole-O-methyltransferase /HIOMT/ in the retina and the pineal gland. in: The pineal gland of vertebrates including man. /Ariens-Kappers J., Pévet P. eds./ Progress in Brain Research Vol. 52. pp. 231-239; Elsevier, Amsterdam

Ebels I. /1981/: Pteridines in the pineal organ. in: The pineal organ. Photobiology - Biochronometry - Endocrinology. /Oksche A., Pévet P. eds./ Developments in Endocrinology Vol. 14. pp. 281-302; Elsevier, Amsterdam

Hoffman R.A. /1982/: Seasonal reproductive cycles in golden hamsters: speculations on data and dogma. in: The pineal gland and its hormones. /Reiter R.J. ed./ pp. 153-166. Alan R. Liss Inc. New York

Kitay J.I., Altschule M.D. /1954/: The pineal gland. Harvard Univ. Press, Cambridge, Mass.

Lerner A.B., Case J.D., Takahashi I. /1960/: Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J. biol. Chem. 235: 1992-1997

Moszkowska A. /1965/: Contribution a l'étude de mécanisme de l'antagonism épiphyso-hypophysaire. Progr. Brain Res. 10: 654-676

Orts R.J., Bruot B.C., Sartin J.L. /1980/: Inhibitory properties of a bovine pineal tripeptide, Threonyl-seryl-lysine on serum Follicle-Stimulating Hormone. Neuroendocrinology 31: 92-95

Pavel S. /1978/: Arginine vasotocin as pineal hormone. J. Neural Trans. Suppl. 13: 135-155

Pévet P., Haldar-Misra C., Öcal T. /1981/: Effect of 5-methoxy-tryptophan and 5-methoxytryptamine on the reproductive system of the male golden hamster. J. Neural Transm. 51: 303-311

Reiter R.J. /1982/: My odyssey with the pineal gland: a retrospective view. EPSG Newsletter 8: 11-15

Reiter R.J., Vaughan M.K. /1977/: Pineal antigonadotrophic substances: polypeptides and indoles. Life Sci. 21: 159-171

Vaughan G.M., Reiter R.J., Siler-Khodr T.M., Sackman J.W., Allen J.P., Vaughan M.K., Mc Guire W.L., Johnson L.Y., Starr P. /1978/: Influence of pinealectomy on serum estrogen and progesterone levels in blind-anosmic female rats. Experientia 34: 1378-1379

Vivien-Roels B. /1981/: Pineal control of reproduction in non-mammalian vertebrates. in: The pineal organ. Photobiology - Biochronometry - Endocrinology. /Oksche A., Pévet P. eds./ Developments in Endocrinology Vol. 14. pp. 315-334; Elsevier, Amsterdam

Wetterberg L., Eriksson O., Friberg Y., Vangt B. /1978/: A simplified radioimmunoassay for melatonin and its application to biological fluids. Preliminary observations on the half-life of plasma melatonin in man. Clinica Chimica Acta 86: 169-177

Wurtman R.J. /1982/: A personal history of pineal research. EPSG Newsletter 7: 12-19

Young S.N., Anderson G.M. /1982/: Factors influencing melatonin, 5-hydroxytryptophol, 5-hydroxyindole acetic acid, 5-hydroxytryptamine and tryptophan in rat pineal glands. Neuroendocrinology 35: 464-468

MORPHOLOGICAL AND CYTOLOGICAL ASPECTS IN PINEAL RESEARCH

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

THE PINEAL GLAND OF MAMMALS AN ORGAN OR A COMPLEX?

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INTRODUCTION

A review of the pertinent literature published in 1979 stressed that in mammals pineal tissue may be arranged in a complex manner and this, together with other findings, led to the question whether the pineal should be termed a complex rather than an organ (Vollrath 1979). The conclusion then reached was that in some species the morphological complexity alone justifies the use of the term pineal complex.

However, in view of the well-known principle that structure and function are interrelated it was thought necessary to support the data relating to the morphological complexity with functional data, before the concept of a mammalian pineal complex were to be eventually adopted.

I do not want to be misunderstood: In my view it does not matter greatly whether we call the pineal a body, an organ, a complex, a system (Sheridan and Reiter 1970) or simply a gland. However, what does matter is whether we have a concept that is stimulating enough to explore even seemingly marginal morphological differences or details that may eventually turn out to be functionally relevant. At present it appears that, globally, relatively few pineal-related morphological papers are published. As more and more pinealogists study non-laboratory and sometimes "exotic" mammals this leads to the wrong impression that the pineal glands of rats, mice, hamsters etc. are morphologically exhausted. However, when pineal research is not separated from the basic mainstreams of neurobiology and endocrinology, as pointed out by the president of the EPSG (Oksche 1984), many fruitful avenues for indepth research open up.

SUPERFICIAL AND DEEP PARTS OF THE PINEAL GLAND

The most important morphological finding pertinent to the concept of pineal complex or system in mammals was the obervation that in Syrian hamsters the pineal is separated into a large superficial and a small deep pineal (Sheridan and Reiter 1970). This subdivision, which is also seen in rats (Boeckmann 1980), is important as after pinealectomy the deep pineal remains in situ and may continue to secrete melatonin. It is therefore of interest to study what effect superficial pinealectomy has on the deep pineal. In rats it was found that 3 and 6 weeks after removal of the superficial pineal gland the deep pineal decreased in volume as did the

pinealocyte nuclei (Heidbüchel and Vollrath 1983a). By contrast, both these parameters showed an increase in golden hamsters examined 30 and 100 days after superficial pinealectomy (Legait et al. 1979). In view of these divergent results it was of interest to obtain data on the melatonin content of the hamster deep pineal gland after superficial pinealectomy. Rollag and Sheridan (1984) carried out such an experiment and reported that the melatonin content was not elevated and that morphologically the deep pineal gland was unaltered when compared to control animals.

A still unresolved problem is which factors affect the location and the sometimes peculiar shape of the pineal gland. As pointed out previously (Vollrath 1979,1981), in most primates as well as in sheep, goat, hyrax, elephant, horse and most of the marsupials and monotremes studied the pineal has a proximal position, i.e. it lies in the vicinity of the posterior and habenular commissures, representing what has been termed a type A pineal. Especially in pinniped carnivores and rodents there is the remarkable feature that pineal tissue is not restricted to the commissural region but is elongated and extends to the cerebellum and sometimes lies directly beneath the skull. In the rodents investigated the distal, superficial part of the pineal complex appears to be the most consistent one, as exemplified by rat, white-footed mouse, hamster, pocket gopher and Dipodomys (Vollrath 1981). It can be regarded as the pineal organ proper in these species. The guinea-pig has an interesting pineal inasmuch as it is dumbbell-shaped and exhibits a number of features that call for a functional interpretation. In two independent studies (Becker and Vollrath, 1983: volumetry; Welker and Vollrath, 1984: protein measurements) it was found that in Pirbright White guinea-pigs the proximal thickening was the largest of the three parts of the pineal complex, followed by the distal thickening and the thin intermediate region.

At the histological level an interesting feature of the guinea pig pineal is a distinct rosette-like arrangement of pinealocytes, which is normally a rare event in rodent pineals. Rosettes are not evenly distributed in the parenchyma. They were found to be absent in the proximal part and a small ventral zone of the distal part, being very conspicuous in the thin intermediate part and the remainder of the distal part (Jung and Vollrath 1982). Differences in a proximo-distal direction are also seen with respect to pinealocytes with hyperchromatic nuclei and a distinctly eosinophilic cytoplasm. These cells are absent in the proximal part of the gland; in the intermediate part they are rare, and are most abundant in the distal area, especially in the ventral zone lacking rosettes. Concretions, which in some species have been related to the secretory activity of the pinealocytes (Reiter et al. 1976) are restricted to the intermediate region where they lie directly beneath the organ's capsule (Jung and Vollrath 1982). At the ultrastructural level it was found that in Syrian golden hamsters synaptic ribbon fields (SRF) were fewer in number in the deep than in the superficial pineal gland. Functionally, it is of interest that under constant darkness and after re-exposure of the animals to a normal light/dark regimen the SRF showed corresponding numerical changes in the two parts of the pineal complex (Hewing 1980).

Is there any evidence that in the guinea-pig pineal gland the different regions of the pineal complex differ in function or respond differently under experimental conditions? To answer this questions, male guinea-pigs were exposed to continuous illumination for 45 days and the

volumes of the different parts of the pineal gland were determined. It was found that both the proximal and distal parts of the gland decreased in volume by approximately 25 % ($p < 0.01$). The thin intermediate part was not affected, but the pinealocyte nuclei showed a decrease in volume in all three regions (Jung and Vollrath 1982).

In an ultrastructural study dealing with "synaptic" ribbons and spherules in the guinea-pig pineal gland regional differences also became apparent (Vollrath et al. 1983). In agreement with previous studies (Vollrath 1973), it was found that ribbons were equally abundant in the proximal, intermediate and distal regions of the gland. However, the spherules were much more abundant proximally than in the intermediate and distal regions (Vollrath et al. 1983). This finding is of interest as ribbons and spherules are usually not found in one and the same pinealocyte profile, and as ribbons and spherules in the guinea-pig exhibit an inverse day/night rhythmicity (Vollrath et al. 1983). Whereas ribbon numbers are low at daytime and increase at night, spherule numbers are high at day and low at night. These findings, in conjunction with previous electrophysiological observations (Semm and Vollrath 1980) led to the working hypothesis that ribbons may characterise nocturnally active pinealocytes, whereas spherules may be typical of diurnally active cells (Vollrath et al. 1983). According to this hypothesis, diurnally active pinealocytes would be most abundant proximally and it was thought worthwhile to study the 24-hour rhythmicity of melatonin formation in the three pineal regions separately.

In this experiment (Welker and Vollrath 1984) three parameters were studied: serum melatonin levels, pineal serotonin N-acetyltransferase (NAT) activity and pineal melatonin levels. Serum melatonin determinations by means of radioimmunoassay revealed the expected nocturnal increase; however, in contrast to other species the day/night ratio of 1:1.25 is rather low. When the pineal gland as a whole is considered, both pineal NAT and melatonin levels show distinct nocturnal increases. A notable feature again is that day/night differences are rather small, in contrast to most species investigated so far but in accordance with findings by Rudeen et al. (1975). When the proximal, intermediate and distal parts of the pineal complex are considered separately, it can be seen that each of the regions is involved in melatonin synthesis. In each region the nocturnal NAT values are slightly higher than the daytime values. Cosinor analysis of NAT activities of two 24-hour experiments revealed in a November experiment an acrophase at 04:00 h in the proximal region and a lack of acrophases in the intermediate and distal regions. In an August experiment acrophases were found at 02:05 h in the proximal region and at 01:30 h in the intermediate and distal regions. On the whole it would appear that the 24-hour oscillations are largest in the proximal part of the pineal gland.

Melatonin determinations in the pineal revealed a first peak at the end of the light period in the proximal and intermediate regions. In all three regions a distinct additional trough was seen at 22:00 h, giving the impression of a bimodal 24-hour curve. Cosinor analysis yielded acrophases at 02:10 h in all three regions.

A comparison of the rhythmicities of pineal NAT activity and melatonin content reveal that the different regions are not always in unison. Whether these differences are functional or dependent on the methodology used is not known at present. However, with the benefit of hindsight it would appear that, due to the relatively small nocturnal increase

of melatonin formation in guinea-pigs, this species is not ideal for studying the rhythmicities of melatonin formation in different parts of the pineal complex.

Sheridan and Rollag (1983) have been more successful in their attempt to elucidate the rhythmicities of the different components of the pineal complex. In 3-week-old Syrian hamsters they found that both the superficial and deep pineal exhibited identical day/night rhythmicities in melatonin content, i.e. low levels during the day, and increasing levels at night, and that in both regions melatonin levels dropped at night when the animals had been exposed to light for 30 min. The absolute quantities of melatonin in the deep pineal gland are approximately 5 % of those in the superficial. In the rat the volume of the deep pineal is approximately 1.5 - 3 % that of the superficial pineal (Boeckmann 1980). It is slightly larger in hamsters; thus it would appear that in the hamster the melatonin concentrations are about the same in the two components of the pineal complex.

The results obtained in guinea-pigs and Syrian hamsters taken together suggest that as far as melatonin formation is concerned there is virtually no evidence for a functional heterogeneity of the different parts of the mammalian pineal complex.

INNERVATION PATTERNS

A potentially important field that has not yet been studied in detail is the distribution of nerve fibres within the pineal parenchyma. There is now abundant evidence that in mammals two main sources of pineal nerve fibres exist: postganglionic sympathetic fibres coming from the superior cervical ganglia and central, commissure-related fibres. Chemically, this latter group is rather heterogeneous, containing serotonin and a number of different peptides. Interestingly, some of these fibres have been shown to exhibit a preferential distribution within the pineal parenchyma. In the dog pineal gland the application of the Falck-Hillarp technique revealed that nerve fibres exhibiting a blue-green fluorescence due to the presence of catecholamines formed a coarse network in the peripheral portion of the gland, especially ventro-apically, that was denser than in the central region of the gland. Serotonergic fibres which persisted after sympathectomy were present in small numbers and were restricted to the proximal part of the gland. The possible site of origin of these fibres is the raphe nuclei (Matsuura and Sano 1983). Vasopressin-, oxytocin- and LHRH-fibres which reach the dog pineal gland via the posterior commissure lie mainly in the proximal and central part of the organ (Matsuura et al. 1983). In the prosimians Galago and Macaca substance P-containing nerve fibres also reveal an uneven distribution inasmuch as they lie mainly in the periphery of the gland (Ronnekleiv et al. 1984).

Regional differences with respect to nervous components are particularly striking in birds. Analysis of 26 avian species revealed that acetylcholinesterase-positive perikarya and processes are mainly present in the postero-proximal portion of the gland, whereas catecholamine-containing fibres predominated in the distal portion, suggesting that sensory pinealocytes may lie preferentially proximally and secretory pinealocytes distally (Sato and Wake 1983).

CORTEX AND MEDULLA

Finally, the problem of cortex and medulla of the pineal gland should be addressed. This aspect has been reviewed in depth (Vollrath 1979) so that it suffices to deal with the more recent findings. As you will recall it has been repeatedly described that in the rat nuclear size of peripheral pinealocytes is larger than that of central pinealocytes. Systematic studies (Heidbüchel and Vollrath 1983b) have revealed that these differences are to a large extent artefactual for a number of reasons. First, the plane of sectioning plays a role. In three series of experiments in which the pineal glands were cut in sagittal, frontal or horizontal planes it was found that in five different regions of the gland's periphery nuclear profile areas differed significantly depending on the plane of sectioning; such differences were not seen in the central region of the gland. As the difference in size between peripheral and central regions was larger when the respective peripheral area was cut more or less tangentially, this indicates that in the periphery of the gland the pinealocyte nuclei are slightly flattened, the flattened side lying parallel to the surface of the gland.

Further experiments revealed that when the isolated pineal glands were bisected in a midsagittal plane prior to immersion fixation and compared with non-bisected glands, nuclear size increased in the centre abolishing the differences between centre and periphery. The differences were also abolished by perfusion fixation. These results indicate clearly that a number of preliminary tests have to be carried out before karyometry in the rat pineal gland can be applied in a meaningful manner.

That important functional differences may exist between pinealocytes located in the periphery and the centre of the pineal gland has been demonstrated in behavioural studies involving psycho-sociological stress (Heinzeller 1981). In these studies exlusively perfusion fixation was applied. The applied stressors varied depending on the species. In the mouse, Mus musculus, density stress was applied, i.e., animals were kept singly and in groups of 3, 9 and 26 respectively for approximately 30 days, starting at the age of 51 - 59 days and 121 - 124 days, respectively. In the cortical region no statistically significant differences of nuclear and nucleolar sizes between the different groups were noted. However, in the centre of the organ both in the subadult and adult groups the nuclei and nucleoli were significantly different in size depending on the number of animals caged together.

In the gerbil, Meriones shawi, psychic castration was induced by not separating the members of a family. This leads to a state in which some adult animals, due to social factors, exhibit signs of reproductive immaturity. In these animals, the pinealocytes of the organ periphery exhibited enlarged nucleoli when compared with mature adults, whereas those of the organ centre showed nuclei and nucleoli diminished in size.

In Tupaia belangeri males kept in pairs for 50 days, the nuclei of marginal pinealocytes increased in size when compared to singly-kept controls, and dominant animals had larger nuclei than subdominant ones. However, in the organ centre pinealocyte nuclear size was indistinguishable between control and dominant animals; in subdominant animals nuclear size decreased significantly both after 3 and 50 days of psycho-sociological stress.

These results show clearly that under certain conditions differences between cortical and medullary pinealocytes may become apparent and that

the concept of a possible subdivision of the pineal parenchyma into cortex and medulla is far from obsolete.

CONCLUSION

Returning to the originally posed question as to whether the pineal gland in mammals is an organ or a complex, I feel that with respect to answering it we are now in an only slightly better position than in 1978. At the gross anatomical level, the sometimes complex arrangement of pineal tissue is highly suggestive of the presence of a pineal complex. Functional data supporting this concept are still meagre. Melatonin formation appears not to differ in the different parts of the complex but morphological data support the notion of a functional heterogeneity, at least in some mammals.

REFERENCES

Becker UG, Vollrath L (1983)
24-Hour variation of pineal gland volume, pinealocyte nuclear volume and mitotic activity in male Sprague-Dawley rats. J Neural Transm 56: 211 - 221

Boeckmann D (1980)
Morphological investigation of the deep pineal of the rat. Cell Tissue Res 210: 283 - 294

Heidbüchel U, Vollrath L (1983a)
Pineal complex of rats: Effects of superficial pinealectomy on the deep pineal. Acta anat 117: 165 - 169

Heidbüchel U, Vollrath L (1983b)
Morphological findings relating to the problem of cortex and medulla in the pineal glands of rat and hamster. J Anat 136: 723 - 734

Heinzeller T (1981)
Morphologische Aspekte verhaltensbiologischer Experimente mit antigonadotroper Auswirkung. Habilitationsschrift, Ludwig-Maximilians-Universität München

Hewing M (1980)
Synaptic ribbons in the pineal system of normal and light deprived golden hamsters. Anat Embryol 159: 71 - 80

Jung D, Vollrath L (1982)
Structural dissimilarities in different regions of the pineal gland of Pirbright White guinea-pigs. J Neural Transm 54: 117 - 128

Legait E, Legait H, Chevalier C (1979)
Modifications de l'épiphyse profonde après ablation de l'épiphyse superficielle chez le Hamster doré. C R Soc Biol 173: 725 - 729

Matsuura T, Sano Y (1983)
Distribution of monoamine-containing nerve fibers in the pineal organ of untreated and sympathectomized dogs. Fluorescence- and immunohistochemical studies. Cell Tissue Res 234: 519 - 531

Matsuura T, Kawata M, Yamada H, Kojima M, Sano Y (1983)
Immunohistochemical studies on the peptidergic nerve fibers in the pineal organ of the dog. Arch Histol Jap 46: 373 - 379

Oksche A (1984)
Letter from the President. EPSG newsletter No. 11: 2

Reiter RJ, Welsh MG, Vaughan MK (1976)
Age-related changes in the intact and sympathetically denervated gerbil pineal gland. Amer J Anat 196: 427 - 432

Rollag MD, Sheridan MN (1984)
Response of Syrian hamster deep pineal gland to superficial pinealectomy. Anat Rec 208: 151A

Ronnekleiv OK, Hess DL, Resko JA, Naylor BR (1984)
Immunohistochemical studies of developing peptidergic and noradrenergic innervation of the prosimian (Galago crassicaudatus) and the macaque (rhesus) pineal. Anat Rec 208: 151A

Rudeen PK, Reiter RJ, Vaughan MK (1975)
Pineal serotonin-N-acetyltransferase activity in four mammalian species. Neurosci Letters 1: 225 - 229

Sato T, Wake K (1983)
Innervation of the avian pineal organ. A comparative study. Cell Tissue Res 233: 237 - 264

Semm P, Vollrath L (1980)
Electrophysiological evidence for circadian rhythmicity in a mammalian pineal organ. J Neural Transm 47: 181 - 190

Sheridan MN, Reiter RJ (1970)
Observations on the pineal system in the hamster. I. Relations of the superficial and deep pineal to the epithalamus. J Morphol 131: 153 - 161

Sheridan MN, Rollag MD (1983)
Development and melatonin content of the deep pineal gland in the Syrian hamster. Am J Anat 168: 145 - 156

Vollrath L (1973)
Synaptic ribbons of a mammalian pineal gland. Circadian changes. Z Zellforsch 145: 171 - 183

Vollrath L (1979)
Comparative morphology of the vertebrate pineal complex. Progr Brain Res 52: 25 - 38 (1979)

Vollrath L (1981)
The Pineal Organ. Hdb d mikr Anat d Mensch (A. Oksche and L. Vollrath eds), Vol. VI/7 Springer-Verlag: Berlin-Heidelberg-New York

Vollrath L, Schultz RL, McMillan PJ (1983)
"Synaptic" ribbons and spherules of the guinea pig pineal gland: Inverse day/night differences in number. Amer J Anat 168: 67 - 74

Welker HA, Vollrath L (1984)
24-Hour rhythmicity of melatonin formation in different parts of the guinea-pig pineal complex. (in preparation)

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

ULTRASTRUCTURAL STUDY OF THE PINEAL-HYPOTHALAMO-HYPOPHYSEO-GONADAL AXIS IN MAMMALS

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INTRODUCTION

Research conducted in the last two decades has contributed significantly to understanding the role of the mammalian pineal gland. Tremendous advances have been made in recent years in defining the relationship of the pineal gland to the endocrine system. Although the pineal gland has been implicate to interact with various endocrine and non-endocrine organs, the majority of evidence suggests that the pineal secretory products influence the structure and function of the reproductive organs via an effect on the hypothalamo-hypophyseo-gonadal axis /Johnson and Reiter 1978, Karasek 1980a, Reiter 1980,1982/. The pineal gland may exert its influence at all levels of the neuroendocrine-reproductive axis /Reiter et al. 1981/. Although the involvement of the hypothalamo-hypophyseo-gonadal system in the feedback mechanism to the pineal gland has been studied rather extensively, many important problems still await elucidation. For example, the question arises, which part, if any, of this system is involved in the feedback relationship to the pineal gland.

This review summarizes the ultrastructural data accumulated on the relationship between the pineal gland and the hypothalamo-hypophyseo-gonadal axis, including the data from a comprehensive research program conducted in our laboratory in regard to the effects of hypothalamic, adenohypophyseal and gonadal hormones on the ultrastructure of rat pinealocytes. Special attention is paid to the ultrastructural correlates of the secretory processes of the mammalian pinealocyte.

ULTRASTRUCTURAL CORRELATES OF THE SECRETORY PROCESSES IN THE MAMMALIAN PINEALOCYTE

Two different secretory processes have been described in mammalian pinealocytes on the basis of ultrastructural studies /Pévet 1977, Pévet and Karasek 1977, Pévet 1979, Karasek 1981, Karasek 1983/. The first process /neurosecretory-like/ is characterized by formation of dense-core vesicles by the Golgi apparatus /Figs. 1, 2, 3/, whereas the

second process /ependymal-like/ is characterized by an accumulation of proteinaceous material in the dilated cisternae of the granular endoplasmic reticulum or by formation from these cisternae of vacuoles containing a flocculent material of moderate electron density /Figs. 1, 4/. Both processes appear to be involved in the production of proteinaceous material /Pévet 1979/.

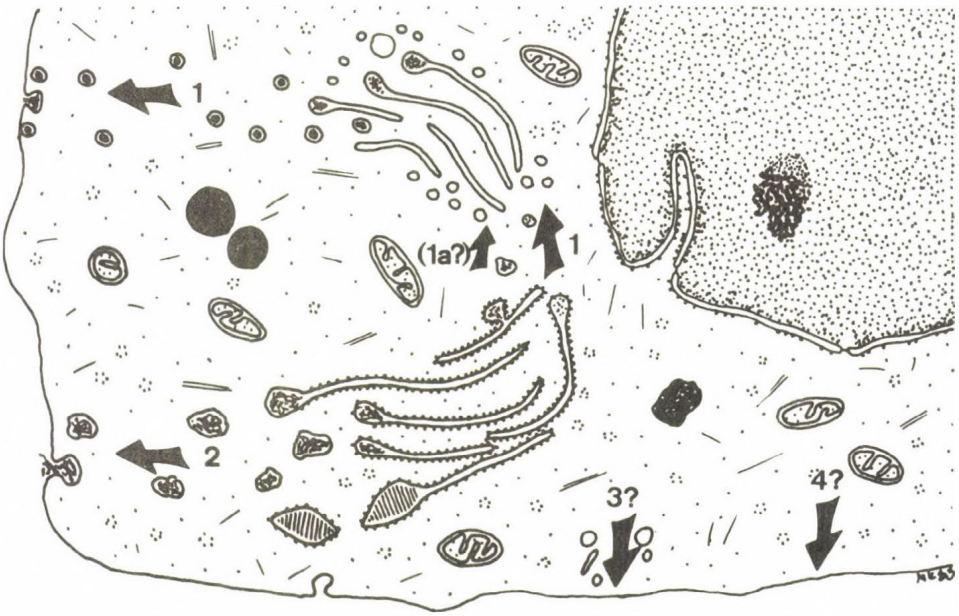

Fig. 1. Putative secretory processes in the mammalian pinealocyte. 1 - neurosecretory-like process, 1a - transitional step in formation of dense-core vesicles /?/, 2 - ependymal-like process, 3 - process involving clear vesicles, 4 - process that cannot be visualized in electron micrographs /From Karasek 1983/.

The question arises whether neurosecretory-like and ependymal-like secretory processes are independent from each other, or whether they are related /e.g., vacuoles containing a flocculent material may represent a transitional step between granular endoplasmic reticulum and Golgi apparatus in the process of the formation of dense-core vesicles; Fig. 1/. On the basis of several experiments /see Karasek 1983/ I believe that neurosecretory-like and ependymal-like secretory processes represent ultrastructural correlates of two different modes of pineal secretion.

It should be mentioned that some authors /Romijn 1973, Welsh et al. 1979, Karasek 1981/ have suggested that clear vesicles may also represent a component of the pineal secretory processes /Figs. 1, 3/. Moreover, the existence of

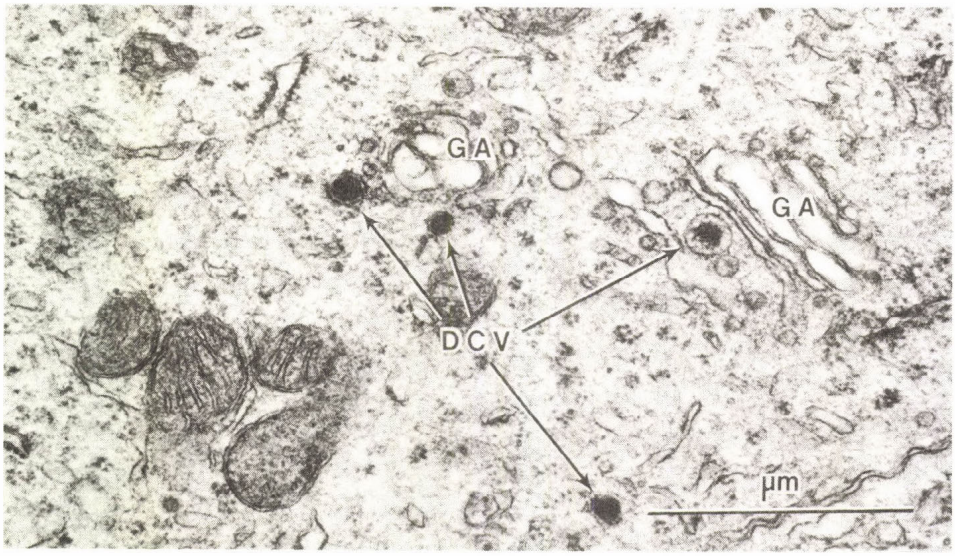

Fig. 2. Ultrastructural correlates of neurosecretory-like secretory process. GA - Golgi apparatus, DCV - dense-core vesicles /Karasek - unpublished micrograph/.

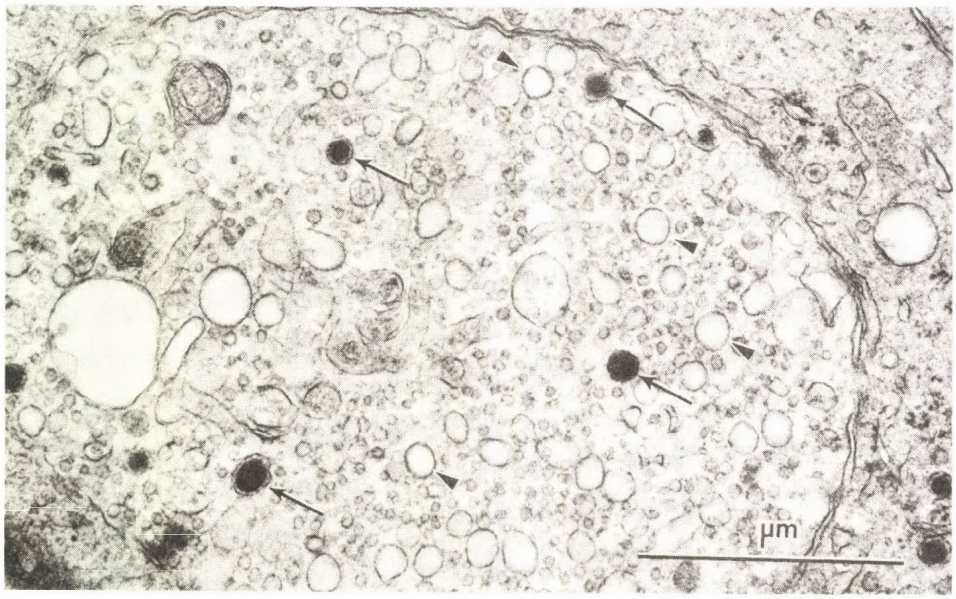

Fig. 3. Dense-core vesicles /arrows/ and clear vesicles /arrow heads/ in terminal of pinealocyte process /Karasek - unpublished micrograph/.

other types of secretory processes that cannot be visualized in electron micrographs /Fig. 1/ should not be ruled out /Reiter 1981/.

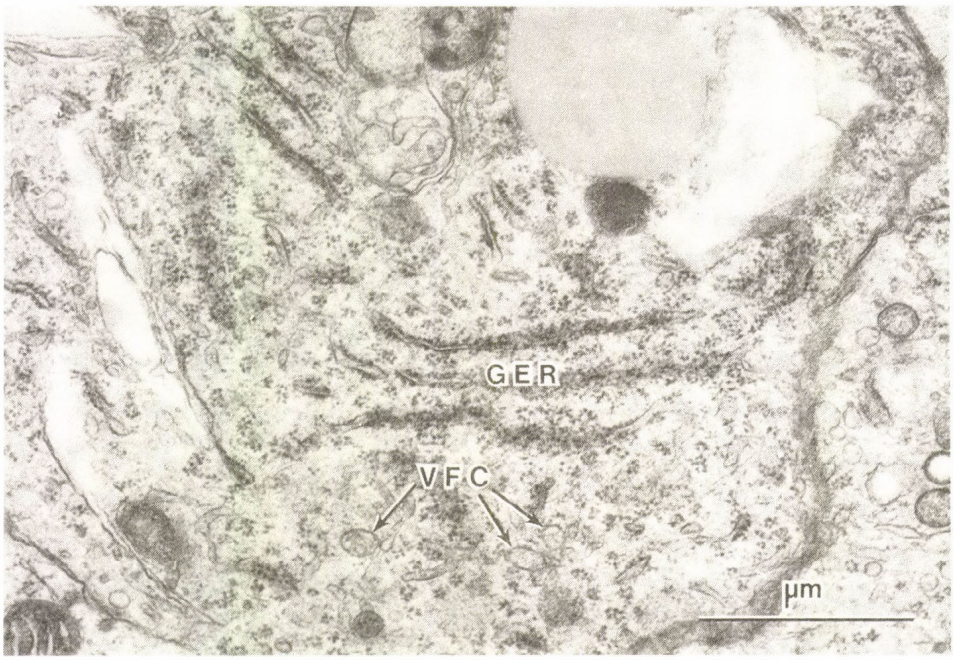

Fig. 4. Ultrastructural correlates of ependymal-like secretory process. GER - granular endoplasmic reticulum, VFC - vacuoles containing flocculent material /Karasek - unpublished micrograph/.

ULTRASTRUCTURAL DATA ON THE PINEAL - HYPOTHALAMO - HYPOPHYSEO - GONADAL RELATIONSHIPS

Pineal and hypothalamus

There is an evidence that the mammalian pineal gland contains LHRH or LHRH-like substances /White et al. 1974, Wheaton 1980, Pévet et al. 1980, Piekut and Knigge, 1981, 1982/, and that LHRH is known to be accumulated in the pineal gland /Redding and Schally 1973, Trentini et al. 1980/. However, only a few ultrastructural studies dealing with the influence of the hypothalamic hormones on the pinealocyte have been published.

It has been shown that administration of LHRH potentiates effects of castration in male rat pinealocytes /Karasek et al. 1976/. Administration of LHRH caused also ultrastru-

ctural changes in pinealocytes of the rabbit /Shiotani 1981/. Haldar-Misra and Pévet /1983a/ have studied in vitro neurosecretory-like secretory process of the pinealocyte in the rat, the hamster and the mouse, and have found that effect of LHRH appears to be species-specific and dependent on the presence or absence of noradrenaline in the culture medium. On the other hand, in the rat, we did not observe ultrastructural changes in the pinealocytes cultured in vitro in the presence of LHRH /Karasek et al. 1978/.

Administration of LHRH in rats at diestrus led to a clear depression of the numbers of both "synaptic" ribbons and spherules, although the mechanism of action of LHRH on these structures is not known /Kosaras et al. 1983a/.

It has been also shown that TRH provoked important changes in the ultrastructure of rat pinealocytes, indicating activation of these cells /Karasek 1980b/. However, we suggested that the ultrastructural changes in pinealocytes caused by TRH may be due to enhanced prolactin levels following TRH treatment /Karasek 1980b/.

Pineal and adenohypophysis

It is well established that the pineal gland influences the synthesis and secretion of hypophyseal hormones /Johnson and Reiter 1978, Reiter 1980, 1982/. However, ultrastructural studies on the influence of the pineal gland or its hormones on the adenohypophysis are very rare. Most of the morphological studies on the relationship between the pineal gland and the adenohypophysis have been concentrated on the effects of hypophyseal hormones on the pinealocyte.

It has been demonstrated that hypophysectomy as well as the administration of gonadotropins or prolactin influences the morphology of pinealocytes /Karasek 1971, 1978, 1979, Karasek and Marek 1978, Karasek et al. 1978, 1982a, 1983, Haldar-Misra and Pévet 1983b/.

In the rat, hypophysectomy caused morphological changes /such as decrease in areas of granular endoplasmic reticulum, lysosomes and lipid droplets/ which indicate diminished activity of pinealocytes /Karasek 1971, Karasek et al. 1982a/. Moreover, signs of diminished activity of the pinealocytes /exemplified by a decrease in areas of mitochondria, granular endoplasmic reticulum, lysosomes, lipid droplets and subsurface cisternae as well as by a decrease in the number of dense-core vesicles/ have been observed in the dwarf mouse having hereditary hypopituitarism /Karasek et al. 1982b/.

It has been demonstrated that co-culture of the rat pineal gland with the adenohypophysis induces activation of the pinealocyte /Karasek 1974/.

In the rat, administration of gonadotropic hormones provoked important changes in the ultrastructure of pinealocytes, either in vivo or in vitro, pointing to an increased synthetic activity of the cells in question /Karasek and Marek 1978, Karasek et al. 1978/. This was exemplified by a conspicious development of the granular endoplasmic reticulum and Golgi apparatus as well as by increased number of

dense-core vesicles, dense bodies, vacuoles containing flocculent material and lipid droplets.

It has been demonstrated that prolactin affects the ultrastructure of the pinealocyte. In the rat, administration of prolactin caused increase in the areas of granular endoplasmic reticulum, vacuoles containing flocculent material and lipid droplets, i.e., changes which can be interpreted as morphological signs of stimulation of pinealocytes /Karasek et al. 1982a/. Moreover, ultrastructural changes which indicate stimulation of the pinealocytes has been found in the rats with chronic hyperprolactinemia induced by pituitary transplants /Karasek et al. 1984/. Administration of prolactin to the hypophysectomized rats reversed some of the effects of hypophysectomy /Karasek et al. 1982a/. In the dwarf mouse, pituitary grafts /which are known to cause an increase in prolactin levels/ as well as administration of prolactin induced activation of pinealocytes /Karasek et al. 1982b, 1983/.

Prolactin has been also shown to influence in vitro neurosecretory-like secretory process in the mouse and in the rat, although the effects depended on the presence or absence of noradrenaline in the culture medium /Haldar-Misra and Pévet 1983b/. Interpretation of the results obtained by Haldar-Misra and Pévet /1983b/ is, however, difficult. It should be stressed that, in the rat, prolactin exerted the stimulatory effect on the ependymal-like secretory process both in vivo /Karasek et al. 1982a/ and in vitro /Haldar-Misra and Pévet 1983b/.

In the rat, blinding and olfactory bulbectomy, the procedures which activate the pineal gland resulted in a marked decrease in the size of the adenohypophysis concomitant with a reduction in the number and size of prolactin cells /Leadem and Blask 1982a/. The prolactin cells of blind-anosmic rats exhibited scant arrays of granular endoplasmic reticulum, small Golgi complexes with few immature secretory granules, few mature secretory granules and rare exocytosis /Leadem and Blask 1982a/. Pinealectomy tended to reverse the effects of blinding and anosmia on prolactin cells size and ultrastructure /Leadem and Blask 1982a/. Leadem and Blask /1982a/ concluded that the pineal gland causes hypotrophy and hypoplasia of prolactin cells in blind-anosmic female rats. These findings correlate well with decreased circulating prolactin levels in blind-anosmic rats /Leadem and Blask 1981, 1982b/. On the contrary, Shiino et al./1974/ reported that prolactin cells of blind-anosmic rats were highly active, and serum prolactin levels were elevated.

In the rabbit, administration of melatonin or arginine vasotocin caused ultrastructural signs of enhanced secretory activity of prolactin cells, and signs of suppressed secretory activity of gonadotropic cells /Shiotani 1981/.

Pineal and gonads

Ultrastructural studies of the pineal-gonadal relationship have been concentrated mostly on the influence of castration or administration of gonadal hormones on the

pinealocyte. Both castration and gonadal hormones influence morphology of pinealocytes.

Activation of the rat pinealocytes by gonadectomy has been reported in a number of studies /Clementi et al. 1965, Gusek and Buss 1966, Bostelmann 1969, Karasek 1976a, Karasek et al. 1976, Ruiz-Navarro et al. 1982/. This is especially marked with reference to the increased development of granular endoplasmic reticulum and Golgi apparatus as well as to the increased number of lysosomes, lipid droplets, vacuoles containing flocculent material and "synaptic" ribbons. The changes were more pronounced when gonadectomy was combined with administration of gonadotropins /Gusek and Buss 1966/ or LHRH /Karasek et al. 1976/. Similar ultrastructural pattern has been observed following castration in the garden dormouse /Roux and Richoux 1981/.

Steroid hormones have been shown to affect various aspects of pineal metabolism /see Cardinali 1981/. However, ultrastructural studies on the influence of estradiol and testosterone on the pinealocyte are rare and inconsistent.

In female rats, administration of estradiol depressed pinealocyte organelles /Clementi et al. 1965/. In contrast, in male rats, large dose of estradiol caused ultrastructural signs of increased activity of the pinealocyte /Karasek and Marek 1980a/. Karasek and Marek /1980a/ raised, however, the question whether the activation of pinealocytes is due to estradiol itself or whether in may be a result of increased prolactin level following estradiol administration. Although estradiol has been shown to increase the number of pineal "synaptic" ribbons /Ruiz-Navarro et al. 1982/, systematic studies of structures in question did not show significant differences in their numbers at the different stages of the estrous cycle /Kosaras et al. 1983b/.

In the rat, testosterone had no effect on the pinealocyte ultrastructure in vivo /Karasek and Marek 1980b/. However, testosterone induced a significant increase in the number of dense-core vesicles with parallel increase in numbers of polyribosomes and cisternae of granular endoplasmic reticulum in the rat and in the mouse pineals cultured in vitro in a noradrenaline-free medium /Haldar-Misra and Pévet 1983c/.

Despite the fact that the pineal gland possesses receptors for sex steroids, and is regarded as a target organ for sex hormones /Cardinali 1981, Vollrath 1981/ rare morphological studies do not clearly indicate influence of sex steroids on the ultrastructure of the pinealocyte. However, one should keep in mind that according to Cardinali /1981/ "several questions should be raised when dealing with a given pineal metabolic change following hormone /particularly steroid/ injection". It should be considered to what extend the changes observed are indirectly mediated via modification of FSH, LH and prolactin levels following steroid administration, and to what extend effects on the pinealocyte may be mediated by changes in the sympathetic neuronal input to the gland. More data /especially from combined in vivo and in vitro studies/ are needed before one may provide clear-cut answers.

CONCLUDING REMARKS

This paper discusses most of the ultrastructural evidence which indicates relationship between the pineal gland and the hypothalamo-hypophyseo-gonadal axis. Ultrastructural investigations have played an important role among the multidisciplinary studies which in recent years have brough tremendous advances in defining the relationship of the pineal gland to the endocrine system, particularly in reference to reproduction. However, it appears from the recent research that relationship between the pineal gland and the hypothalamo-hypophyseo-gonadal system is very complex.

The existence of a reciprocal relationship between the hypothalamo-hypophyseal axis and the pineal gland has been suggested by us several years ago /Karasek 1976b, Karasek et al. 1976/ and subsequently has been demonstrated /Karasek and Marek 1978, Karasek 1978, 1979, 1981, Karasek et al. 1978, 1982a,b, 1983, Karasek and Reiter 1982/. This relationship may involve an enhancement of the synthetic activity of the pineal gland by gonadotropins or prolactin, leading to an increase in the elaboration of pineal antigonadotropic and/or prolactin-regulating factors. Both secretory processes of the pinealocyte, i.e., neurosecretory-like and ependymal-like seem to be involved in this relationship. It has been suggested that both secretory processes are involved in different regulatory mechanisms in pinealocytes /Karasek et al. 1982a/. Some data /Karasek et al. 1982a,b, 1983/ indicate that ependymal-like secretory process may be related to the regulation of prolactin synthesis and/or secretion. However, at present, it is not possible to establish which pineal putative hormones may be involved in this regulation.

The existence of a reciprocal relationship between the pineal gland and the hypothalamo-hypophyseal system do not exclude the possibility that a feedback mechanism also exists between other parts of the hypothalamo-hypophyseo-gonadal system and the pineal gland, especially considering the fact that the organ probably secretes a number of structurally diverse active compounds.

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REFERENCES

Bostelmann W. /1969/ Der Einfluss der bilateralen Kastration auf die Feinstruktur der Zirbeldrüse. Endokrinologie, 54:56-65.

Cardinali D.P. /1981/ Hormone effects on the pineal gland. In: The Pineal Gland, Vol.1, Anatomy and Biochemistry, R.J.Reiter, ed., CRC Press, Boca Raton, pp. 243-272.

Clementi F., Fraschini F., Muller E., Zanoboni A. /1965/ The pineal gland and the control of electrolyte balance and

of gonadotropin secretion: functional and morphological observations. Progr. Brain Res., 10:583-603.
Gusek W., Buss H. /1966/ Morphologische und histochemische Veränderungen der Zirbeldrüse unter dem Einfluss von Prolan und nach Ovariektomie. Frankfurt. Z. Path., 75:172-186.
Haldar-Misra C., Pévet P. /1983a/ The influence of luteinizing hormone-releasing hormone /LHRH/ on the process of protein and/or peptide secretion characterized by the formation of granular vesicles in mammalian pinealocytes. Cell Tissue Res., 232:529-538.
Haldar-Misra C., Pévet P. /1983b/ Influence of prolactin on the process of protein and/or peptide secretion in mouse and rat pinealocytes. An in vitro study. J. Neural Transm., 58:245-259.
Haldar-Misra C., Pévet P. /1983c/ Influence of testosterone on protein/peptide secretion as characterized by the formation of granular vesicles in rat and mouse pinealocytes. An in vitro study. J. Neural Transm., 57:111-120.
Johnson L.Y., Reiter R.J. /1978/ The pineal gland and its effects on mammalian reproduction. Progr. reprod. Biol., 4:116-156.
Karasek M. /1971/ Ultrastructure of the epiphysis in white rats under normal conditions and after hypophysectomy. Pol. Endocrinol., 22:13-26.
Karasek M. /1974/ Ultrastructure of rat pineal gland in organ culture; influence of norepinephrine, dibutyryl cyclic adenosine 3',5' - monophosphate and adenohypophysis Endokrinologie, 64:106-114.
Karasek M. /1976a/ Quantitative changes in number of "synaptic" ribbons in rat pinealocytes after orchidectomy and in organ culture. J. Neural Transm., 38:149-157.
Karasek M. /1976b/ The Pineal Gland. PZWL, Warszawa /in Polish/.
Karasek M. /1978/ Pineal-adenohypophysial relationships in the rats. An ultrastructural contribution. Endokrynol. Pol., 29:371-379.
Karasek M. /1979/ Ultrastructural study of the pineal-adenohypophysial relationships in rats. Progr. Brain Res., 52:195-198.
Karasek M. /1980a/ The pineal gland and the hypothalamo-hypophysial axis. In: Peptides and Brain Function, M.Karasek and M.Pawlikowski, eds., Acad. Med. Lodz, Lodz, pp. 131-145.
Karasek M. /1980b/ Influence of TRH and TSH on the ultrastructure of rat pineal gland. Acta Med. Pol., 21: 347-348.
Karasek M. /1981/ Some functional aspects of the ultrastructure of rat pinealocytes. Endocrinol. Exp., 15:17-34.
Karasek M. /1983/ Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. In: Pineal Research Reviews, R.J.Reiter, ed., Vol. 1, Alan R.Liss, New York, pp. 1-48.
Karasek M., Marek K. /1978/ Influence of gonadotropic hormones on the ultrastructure of rat pinealocytes. Cell Tissue Res., 188:133-141.

Karasek M., Marek K. /1980a/ Influence of estradiol on the ultrastructure of rat pineal gland. Acta Med. Pol., 21:355-356.

Karasek M., Marek K. /1980b/ Ultrastructure of the rat pineal gland after testosterone administration. Acta Med. Pol., 21:349-350.

Karasek M., Reiter R.J. /1982/ A reciprocal relationship between the adenohypophysis and the pineal gland. Med. Hypotheses, 9:1-9.

Karasek M., Pawlikowski M., Kappers J. Ariëns, Stępień H. /1976/ Influence of castration followed by administration of LH-RH on the ultrastructure of rat pinealocytes. Cell Tissue Res., 167:325-339.

Karasek M., Marek K., Kunert-Radek J. /1978/ Ultrastructure of rat pinealocytes in vitro: influence of gonadotropic hormones and LH-RH. Cell Tissue Res., 195:547-556.

Karasek M., Lewinski A., Hansen J.T., Reiter R.J. /1982a/ Influence of hypophysectomy and prolactin on the rat pinealocyte: a quantitative ultrastructural study, Reprod. Nutr. Develop., 22:785-792.

Karasek M., Bartke A., King T.S., Hansen J.T., Reiter R.J. /1982b/ Effects of hereditary hypopituitarism and ectopic pituitary transplants on pinealocytes of the mouse: a quantitative ultrastructural study. Endocrinology, 110:2183-2185.

Karasek M., Bartke A., Hansen J.T. /1983/ Influence of prolactin on pinealocytes of the mouse with hereditary hypopituitarism: a quantitative ultrastructural study. Mol. Cell. Endocrinol., 29:101-108.

Karasek M., Bartke A., Doherty P.C. /1984/ Effects of experimentally-induced chronic hyperprolactinemia on the ultrastructure of pinealocytes in male rats. J. Pineal Res., in press.

Kosaras B., Welker H.A., Mess B., Vollrath L. /1983a/ Depressive effect of LHRH on the number of "synaptic" ribbons and spherules in the pineal gland of diestrous rats. Cell Tissue Res., 229:461-466.

Kosaras B., Welker H.A., Vollrath L. /1983b/ Pineal "synaptic" ribbons and spherules during the estrous cycle in rats. Anat. Embryol., 166:219-227.

Leadem C.A., Blask D.E. /1981/ Evidence for an inhibitory influence of the pineal on prolactin in the female rat. Neuroendocrinology, 33:268-275.

Leadem C.A., Blask D.E. /1982a/ Immunocytochemical and ultrastructural correlates of the pineal inhibition of prolactin cell activity in blind-anosmic female rats. Cell Tissue Res., 227:343-350.

Leadem C.A., Blask D.E. /1982b/ A comparative study of the effects of the pineal gland on prolactin synthesis, storage and release in male and female blind-anosmic rats. Biol. Reprod., 26:413-421.

Pévet P. /1977/ On the presence of different populations of pinealocytes in the mammalian pineal gland. J. Neural Transm., 40:289-304.

Pévet P. /1979/ Secretory processes in the mammalian pinealocyte under natural and experimental conditions. Progr.

Brain Res., 52:149-192.
Pévet P., Karasek M. /1977/ Are the pineal active compounds of mammals proteinaceous in nature? An ultrastructural contribution. Acta Med. Pol., 18:351-353.
Pévet P., Ebels I., Swaab D.F., Mud M.T., Arimura A. /1980/ Presence of AVT-, α-MSH-, LHRH- and somatostatin-like compounds in the rat pineal gland and their relationship with the UM05R pineal fraction. An immunocytochemical study. Cell Tissue Res., 206:341-353.
Piekut D., Knigge K.M. /1981/ Immunohistochemical analysis of the rat pineal gland using antisera generated against luteinizing hormone-releasing hormone /LHRH/. J. Histochem. Cytochem., 29:616-622.
Piekut D., Knigge K.M. /1982/Immunohistochemical analysis of the rat pineal gland using antisera generated against analogs of luteinizing hormone-releasing hormone /LHRH/. J. Histochem. Cytochem., 30:106-110.
Redding T.W., Schally A.V. /1973/ The distribution, halflife and excretion of tritiated luteinizing hormone-releasing hormone /LHRH/ in rats. Life Sci., 12:23-25.
Reiter R.J. /1980/ The pineal and its hormones in the control of reproduction in mammals. Endocrinol. Rev., 1:109-131.
Reiter R.J. /1981/ The mammalian pineal gland: structure and function. Am. J. Anat., 162:287-313.
Reiter R.J. /1982/ Neuroendocrine effects of the pineal gland and melatonin. In: Frontiers in Endocrinology, Vol. 7, W.F.Ganong and L.Martini, eds., Raven Press, New York, pp. 301-330.
Reiter R.J., Richardson B.A., Vaughan M.K., Johnson L.Y. /1981/ Pineal actions and mechanisms in reproductive physiology. Jikeikai Med. J., 28 /Suppl.1/:31-46.
Romijn H.J. /1973/ Structure and innervation of the pineal gland of the rabbit, Oryctolagus cuniculus /L./. II. An electron microscopic investigation of the pinealocytes. Z. Zellforsch., 141:545-560.
Roux M., Richoux J.P. /1981/ Effect of castration and adrenalectomy on the ultrastructure of pinealocytes in the female garden dormouse /Eliomys quercinus L./ under different conditions of lighting and temperature during the normal period of hibernation. J. Neural Transm., 51:283-294.
Ruiz-Navarro A., Blanco-Rodriguez A., Gazquez-Ortiz A., Jover-Moyano A. /1982/ Synaptic ribbons in pinealocytes of castrated rats and rats treated with estradiol. Cell Biol. Int. Rep., 6:629-633.
Shiino M., Arimura A., Rennels E.G. /1974/ Effects of blinding, olfactory bulbectomy, and pinealectomy on prolactin and growth hormone cells of the rat with special reference to ultrastructure. Am. J. Anat., 139:191-207.
Shiotani Y. /1981/ Interactions between the pineal gland and the hypothalamo-adenohypophysial system. An ultrastructural study. Jikeikai Med. J., 28 /Suppl.1/:53-64.
Trentini G.P., De Gaetani C.F., Di Gregorio C., Botticelli C.S. /1980/ LHRH incorporation in normal and denervated pineal gland and in pineal glands of rats with

constant estrous-anovulatory syndrome: a preliminary study. Endokrinologie, 76:6-12.
Vollrath L. /1981/ The Pineal Organ, Springer-Verlag, Berlin.
Welsh M.G., Cameron I.L., Reiter R.J. /1979/ The pineal gland of the gerbil, Meriones unguiculatus. II. Morphometric analysis over a 24-hour period. Cell Tissue Res., 204: 95-109.
Wheaton J.E. /1980/ Immunoreactive luteinizing hormone-releasing hormone /LHRH/ in ovine pineal glands. Horm. Metab. Res., 12:314-317.
White W.F., Hedlund M.T., Weber G.F., Rippel R.H., Johnson E.S., Wilber J.F. /1974/ The pineal gland: a supplemental source of hypothalamic-releasing hormones. Endocrinology, 94:1422-1426.

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The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

THE CENTRAL INNERVATION OF THE MAMMALIAN PINEAL ORGAN

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INTRODUCTION.

The pineal organ of vertebrates, a derivative of the neuroepithelium, is an integral component of the central nervous system. In poikilothermic species the pineal complex is endowed with a prominent neuronal apparatus providing a direct neural communication between the pineal and the brain (Oksche 1983, 1984). Central nervous connections are also shown in different avian species (cf. Korf et al. 1982; Korf and Vigh-Teichmann 1984). The application of modern tracer techniques reveals that the vast majority of nerve fibers constituting the pineal tract runs in pinealofugal direction and terminates in different regions of the diencephalon and upper brainstem (Eldred et al. 1980; Korf and Wagner 1981; Ekström and van Veen 1983; Ekström 1984). However, in lizards and house sparrows nerve fibers originating from perikarya in the brain intermingle with pinealofugal axons (Korf and Wagner 1981; Korf et al. 1982). Apparently, the number of pinealopetal nerve fibers of central origin increases during phylogeny from lacertilians to birds.

The innervation of the mammalian pineal organ must be viewed in context with these comparative data. During ontogeny, an unpaired midsagittal nerve is observed connecting the pineal organ with the rostralmost part of the mesencephalic tectum in the human fetus, fetal rabbit and sheep (Møllgard and Møller 1973; Møller 1978, 1979; Møller et al. 1975). Neuroblasts endowed with axosomatic and axodendritic synapses are associated with these pathways which, in older fetuses, are gradually incorporated into the pineal parenchyma. Probably, this pathway is homologuous to the midsagittal pineal tracts of poikilothermic vertebrates (Møller 1979) and thus may be composed predominantly of pinealofugal fibers. The presence of neuroblasts within the fetal nerve might point toward a sensory capacity of the mammalian pineal organ during embryonic development comparable to the photoreceptive capacity of the pineal complex in poikilothermic vertebrates (Dodt 1973; Meissl and Dodt 1981). This assumption is supported by the demonstration of extraretinal photoreceptive elements in newborn rats (Zweig et al. 1966).

The fate of the pineal tract is open to discussion. Since it is no longer detectable at birth one may suggest that these connections undergo degeneration during ontogeny. On the other hand, axons of the pineal tract may be incorporated into the system of nerve fibers connecting the pineal organ to the habenular or posterior commissures.

Nerve fibers emerging from the habenular or posterior commissures and entering the pineal organ were shown to persist in adult individuals of several mammalian species, including monkeys and humans, by means of silver impregnations of the Bodian or Palmgren type (dog: Hosaka et al. 1957; cat: Nielsen and Møller 1975; monkey: Hosaka et al. 1957; Nielsen and Møller 1975; human: Scharenberg and Liss 1965). However, this method did not allow to analyze terminal formations of the impregnated nerve fibers. Moreover, in the rat (Kappers 1960, 1965), rabbit (Romijn 1973) and the monkey (Le Gros Clark 1940) some of these axons represent aberrant elements of the commissural systems.

Thus, the central neural projections to the pineal organ of adult mammals remained enigmatic for a long period in contrast to the sympathetic innervation in lesion experiments proven to exist in the rat by Kappers (1960, 1965). However, there is now accumulating evidence for the existence of direct neural connections between the brain and the pineal organ in different mammalian species. Data supporting the presence of these connections have been derived from both electrophysiological and morphological investigations (cf. Korf and Møller 1984). The electrophysiological results have been recently reviewed by Semm (1981, 1983); the present contribution will concentrate on structural findings concerned with central connections of the mammalian pineal organ.

The following aspects will be analyzed in this review:

1. Do nerve fibers occurring in the pineal stalk represent central nervous elements or are they sympathetic nerve fibers traversing the pineal and finally innervating the habenular complex as was shown in the rat (Björklund et al. 1972; Wiklund 1974)?
2. In which direction do these fibers run?
3. What is known about the nature of terminal formations of central pinealopetal projections?
4. Where do pinealopetal nerve fibers of central origin come from?
5. Which transmitters are characteristic of the central nervous connections of the pineal organ?
6. What is the evidence for the existence of pinealofugal nerve fibers of central origin in mammals?

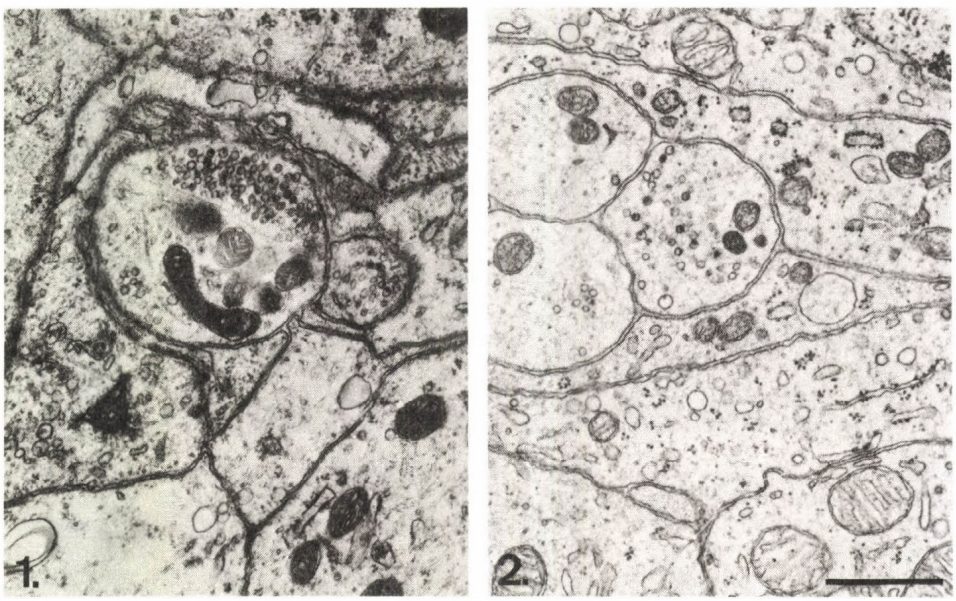
THE ULTRASTRUCTURE OF INTRAPINEAL TERMINAL FORMATIONS

At the electron-microscopic level, nerve fibers penetrating into the pineal stalk of the golden hamster (Hewing 1976), the guinea pig (Lues 1971; Schneider et al. 1981), the Dsungarian hamster (Ueck 1979), the rat (Pfister et al. 1978;

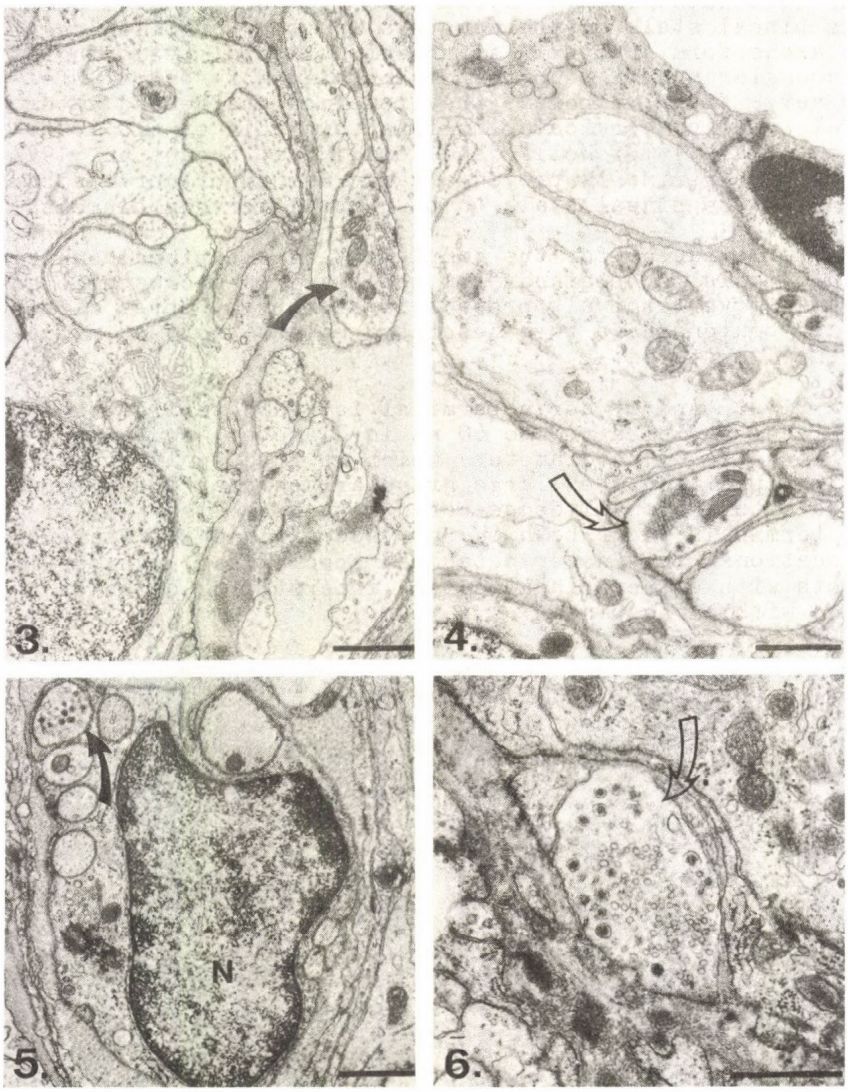
Zuo et al. 1984), and the Mongolian gerbil (Møller and Korf 1983a) were identified as myelinated and unmyelinated elements. In the pineal stalk of the hamsters and the Mongolian gerbil these axons form tracts of different calibers. Lesioning of the habenular nuclei prompted degeneration of several of these nerve fibers, whereas they remained unaffected after bilateral superior cervical ganglionectomy (Lin et al. 1975; Pfister et al. 1978; Møller and Korf 1983a; Zuo et al. 1984). The lesion experiments proved a large number of nerve fibers running in the pineal stalk to be central nervous elements.

Further evidence for the complexity of the pineal innervation in mammals is obtained from the electron-microscopic analysis of nerve terminals present in the organ. Three different types of boutons have been described on the basis of ultrastructural criteria:

The first type of nerve terminal is characterized by small transmitter vesicles (40 to 60 nm in diameter) containing a dense core (25 nm in diameter) which is frequently found in an eccentric position. Few large granular vesicles are intermingled with the small dense-cored vesicles (Figs. 1, 2). These terminals occur both in perivascular and intraparenchymal locations. They never establish specialized, synapse-like contacts with either pinealocytes, interstitial cells or neurons within the pineal.



Figs. 1,2. Sympathetic boutons in the superficial pineal of the Mongolian gerbil (Fig. 1) and the rat (Fig. 2). The boutons contain small transmitter vesicles (40 to 60 nm in diameter confining an eccentric dense core (25 nm in diameter). Bar indicates 1 µm.



Figs. 3, 4. Nerve terminals in the superficial portion of the pineal organ of the Mongolian gerbil containing small clear transmitter vesicles 40 to 60 nm in diameter (bended arrows). This type of bouton is unaffected by superior cervical ganglionectomy, but degenerates after lesioning the habenular nuclei.
Figs. 5, 6. Nerve terminals in the superficial portion of the pineal organ of the Mongolian gerbil containing a large number of granular vesicles 100-200 nm in diameter in addition to small, clear transmitter vesicles (40 to 60 nm in diameter). Nucleus (N).
Bar indicates 1 µm.

In the mammalian pineal organ these elements are generally considered as sympathetic nerve fibers (see Korf and Møller 1984, for review). The degeneration of these boutons after bilateral ablation of the superior cervical ganglia in the Mongolian gerbil (Møller and Korf 1983a) conforms to this assumption. However, in the rat, boutons containing small dense-cored vesicles were shown to degenerate in the pineal organ after lesioning of the habenular complex (Rønnekleiv and Møller 1979). Two possible explanations can be offered for this result: (1) In the rat, lesioning of the habenular complex might lead to degeneration of sympathetic nerve fibers involved in the innervation of the medial habenular complex (cf. Björklund et al. 1972; Wiklund 1974). (2) The degenerating boutons may belong to central pinealopetal projections containing biogenic amines as neurotransmitters.

The second type of intrapineal nerve terminal is endowed with numerous small, clear transmitter vesicles (40 to 60 nm in diameter) intermingling with a few large granular vesicles approximately 100 nm in diameter (Figs. 3, 4). As has been shown in the monkey (David et al. 1975), ferret (David and Herbert 1973; David et al. 1973) and the Mongolian gerbil (Møller and Korf 1983a) these boutons degenerate after habenular lesions and, thus, belong to central pinealopetal nerve fibers. Most of these boutons end freely in the perivascular space or in the pineal parenchyma (Korf and Møller 1984); however, in several species they are occasionally shown to establish synaptic contacts with pinealocytes or intrapineal neurons. The functional significance of the latter, see below. At this point it should be only emphasized that the number of intrapineal neurons exhibits a considerable interspecific variation (see Korf and Møller 1984, for references).

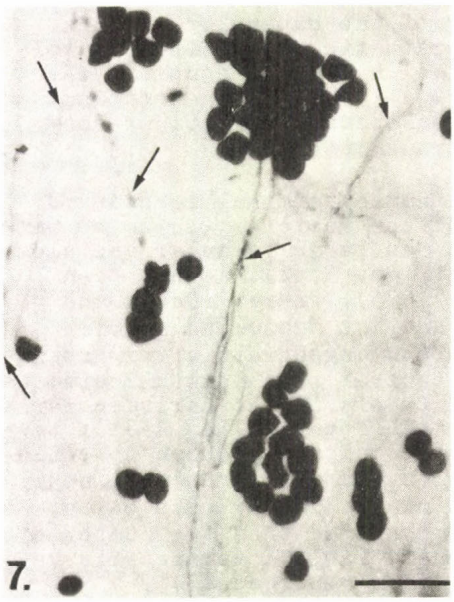
The third type of intrapineal nerve terminal contains numerous large granular vesicles (approximately 100 nm in diameter) intermingling with small clear vesicles (Figs. 5, 6). These terminals closely resemble those in the posterior lobe of the pituitary and may therefore be considered as peptidergic elements (cf. Korf and Møller 1984; Schneider et al. 1981; Zuo et al. 1984).

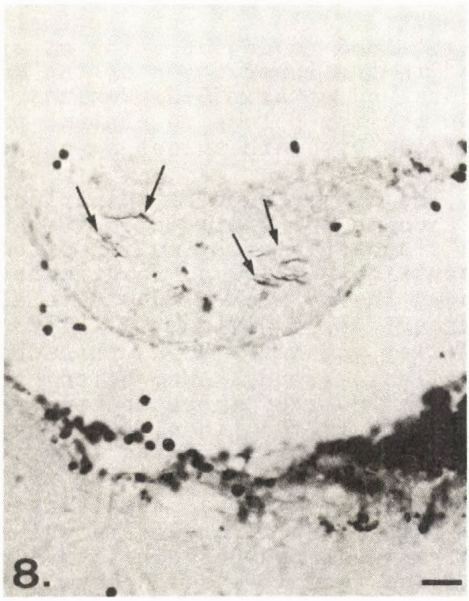
In summary, electron-microscopic investigations have shown that the different types of boutons predominantly terminate freely in the perivascular space of the intrapineal capillaries or between the pinealocytes. Furthermore, the results of these studies lead to the conclusion that a considerable number of nerve fibers in the mammalian pineal organ belongs to pinealopetal systems of central origin.

THE ORIGIN OF CENTRAL PINEALOPETAL PROJECTIONS

The injection of horseradish peroxidase (HRP) into the pineal organ of different rodent species confirms the presence of central pinealopetal nerve fibers. HRP-labelled nerve fibers are demonstrated in the pineal stalk of the guinea pig (Korf and Wagner 1980), rat (Korf and Møller 1983), Mongolian ger-

bil (Møller and Korf 1983b; Fig. 7) and the golden hamster (Fig. 8).





Figs. 7,8. HRP-labelled nerve fibers (arrows) in the pineal stalk of the Mongolian gerbil (Fig. 7) and the deep portion of the pineal organ of the golden hamster (Fig. 8). Two types of nerve fibers can be distinguished with respect to their calibers. Bar indicates 25 µm.

Whereas these fibers form fine tracts in the Mongolian gerbil, in the remaining species they are scattered in different sectors of the pineal stalk. Two types of nerve fibers can be distinguished with respect to their calibers: (1) delicate axons approximately 0.5 µm in diameter, exhibiting numerous local swellings, and (2) relatively thick fibers (1 to 2 µm in diameter) free of swellings (Fig. 7). Both types of fibers were frequently seen to dichotomize in distal direction (toward the superficial portion of the organ). This finding indicates that these nerve fibers represent pinealopetal axons innervating the pineal organ. The fibers taper into the deep portion of the pineal organ (Figs. 9, 10) via either the habenular or the posterior commissures. They were never seen to cross to the contralateral side.

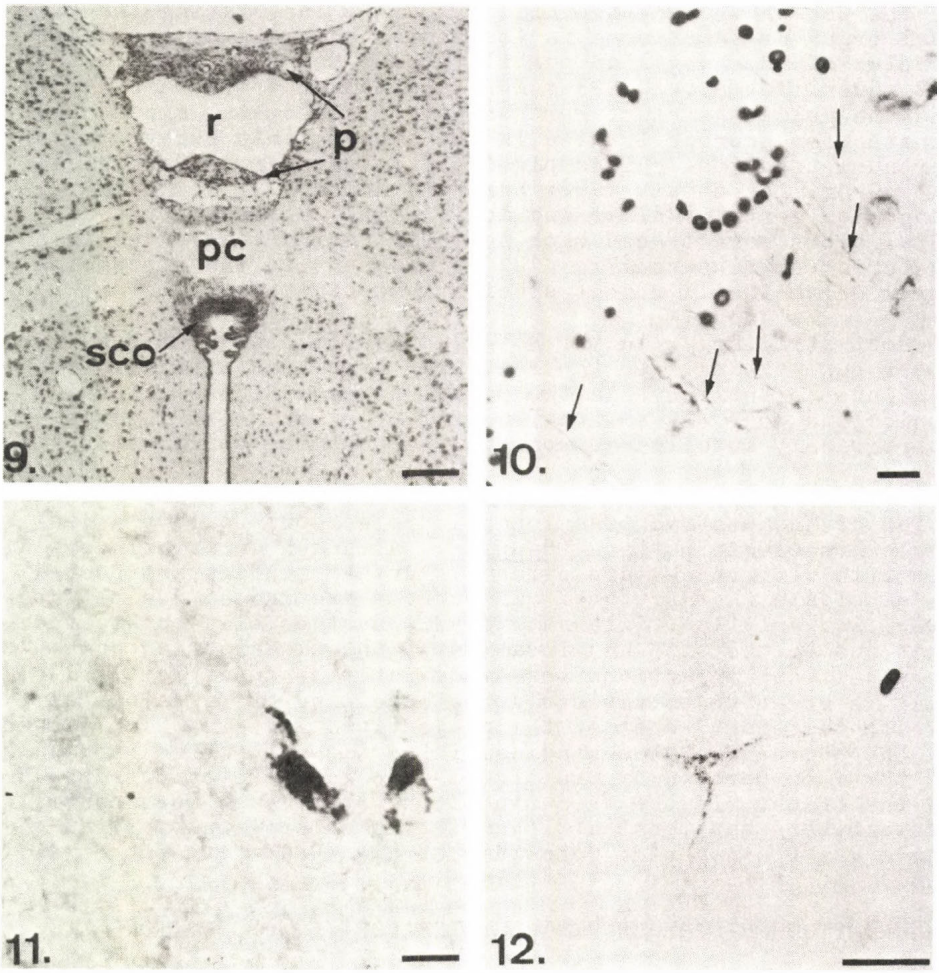


Fig. 9. Frontal section through the deep pineal of the Mongolian gerbil (p) at the level of the posterior commissure (pc). Cresyl violet stain. Pineal recess (r); subcommissural organ (sco). Bar indicates 1 mm.
Fig. 10. Frontal section through the deep pineal of the Mongolian gerbil. HRP-labelled fibers (arrows) can be traced from the deep pineal to the brain. Bar indicates 25 µm.
Fig. 11. Neurons in the paraventricular nucleus of guinea pig retrogradely labelled after injection of HRP into the superficial pineal. Bar indicates 10 µm.
Fig. 12. Neuron located in the nucleus of the posterior commissure retrogradely labelled after the injection of HRP into the superficial pineal of the Mongolian gerbil. Bar indicates 25 µm.

The origin of these nerve fibers can be readily elucidated by the use of the HRP-technique. Neurons innervating the pineal organ are scattered in the medial and lateral habenular complex of the Mongolian gerbil (Møller and Korf 1983b), the rat (Guerillot et al. 1982; Korf and Møller 1983; Dafny 1983) and the golden hamster. Furthermore, pinealopetal axons originate from neurons located in the hypothalamic paraventricular nucleus of the guinea pig (Korf and Wagner 1980; Fig. 11), rat (Guerillot et al. 1982; Korf and Møller 1983) and the Mongolian gerbil (Møller and Korf 1983b). Moreover, pinealopetal axons were traced back to their perikarya located in the posterior commissure (Fig. 12) or adjacent to the subcommissural organ in all rodent species investigated.

Most strikingly, in the Mongolian gerbil, nerve fibers after penetrating into the habenular area bend laterally and continue to perikarya in the dorsal nucleus of the lateral geniculate body. In the rat, Guerillot et al. (1982) found retrogradely labelled neurons in the superior colliculi.

As can be inferred from these data in rodents, the organization of the central pinealopetal projections follows a general morphologic pattern (Bauplan), although certain interspecific differences are evident. Further studies are needed to elucidate whether these differences are indeed due to interspecific variation. Alternatively it must be also considered that due to the size and location of the pineal organ only very delicate injections were made into the superficial pineal in order to obtain exclusive labelling of pinealopetal axons; therefore, some of the projections may have been missed in the above-mentioned studies. In particular, nerve terminals in the deep portion of the pineal were inconsistently exposed to the tracer. In the deep pineal, however, even more central nerve fibers may terminate than in the superficial portion of the organ (guinea pig: Schneider et al. 1981; rat: Zuo et al. 1984).

IMMUNOCYTOCHEMISTRY OF CENTRAL PINEALOPETAL PROJECTIONS

The diversity of the pineal innervation in mammals is also shown by immunocytochemical techniques. Vasopressin- and oxytocin-immunoreactive nerve fibers are found in the pineal organ of the rat (Buijs and Pévet 1980), hedgehog (Nürnberger and Korf 1981; Fig. 16) and dog (Matsuura et al. 1983a). The fibers enter the pineal either via the habenular or the posterior commissures and form terminals predominantly in the perivascular space of pineal capillaries. In the hedgehog, the amount of vasopressin- and oxytocin-immunoreactive material exhibited characteristic seasonal variation different from that observed in the posterior lobe of the pituitary, thus indicating that these pinealopetal projections establish a specialized subunit of the vasopressin- and oxytocin-system (for details, see Nürnberger and Korf 1981; Korf and Møller 1984). The vasopressin- or oxytocin-containing pinealopetal axons of the hedgehog display in addition neurophysin-immunoreactive material. Neurophysin-immunoreactive nerve fibers also occur in the pineal organ of the guinea pig (Weindl and

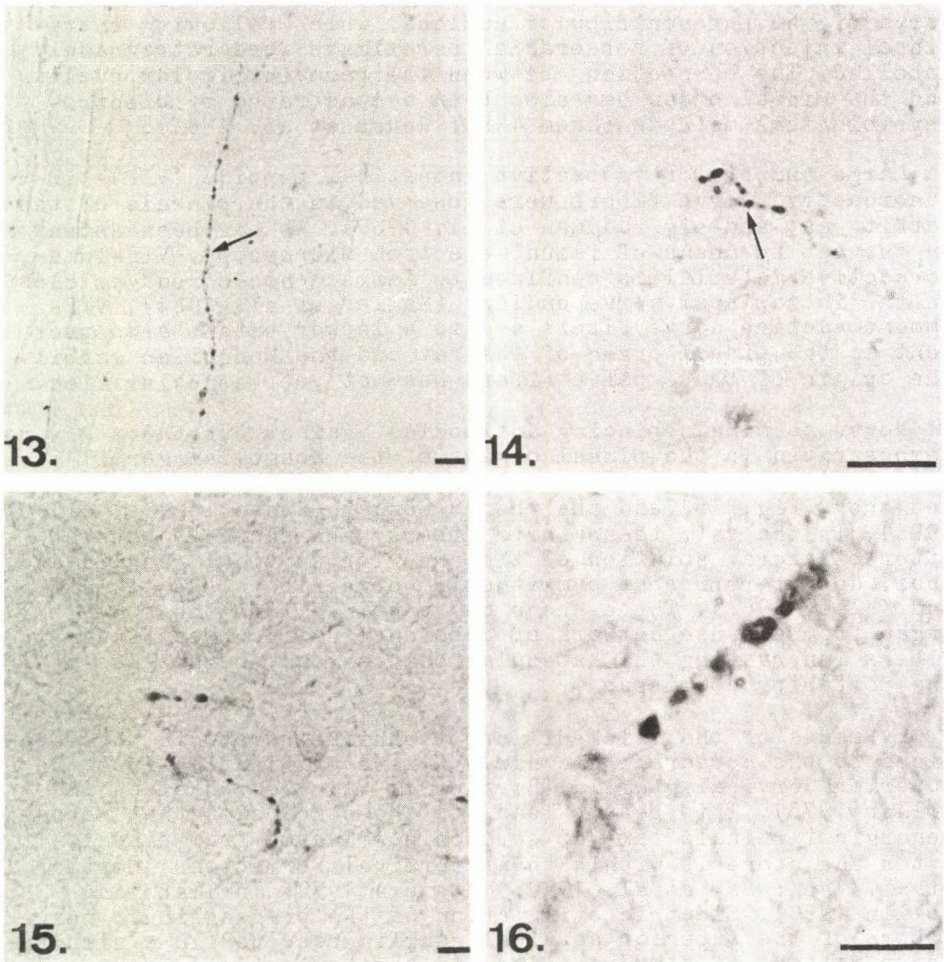


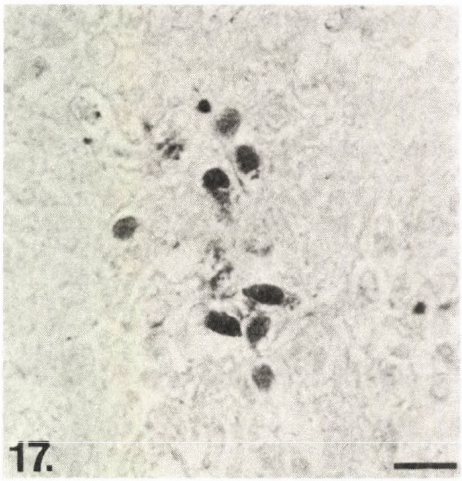
Fig. 13. Sagittal section through the pineal stalk of the Mongolian gerbil. Long nerve fiber displaying substance P-like immunoreactivity (arrow). Bar indicates 10 µm.
Fig. 14. Frontal section through the superficial pineal of the rat. Nerve fibers displaying substance P-like immunoreactivity are located in the perivascular space (arrow). Bar indicates 25 µm.
Figs. 15, 16. Frontal sections through the pineal organ of the hedgehog. Nerve fibers containing substance P-like (Fig. 15) and vasopressin-like (Fig. 16) immunoreactive material. Bar indicates 10 µm.

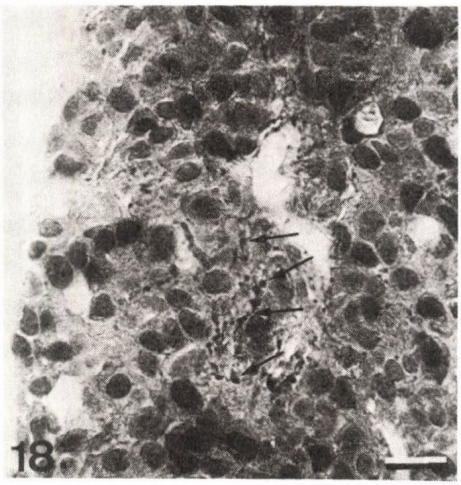
Sofroniew 1982) and the rat (Yulis and Rodríguez 1982). Most probably, these peptidergic projections originate from perikarya of the paraventricular nucleus, which following intrapineal injection of horseradish peroxidase were retrogradely labelled. The connection between the paraventricular nucleus and the pineal organ has also been demonstrated by electrophysiological methods (Semm 1983; Reuss et al. 1984).

Large numbers of vasoactive intestinal peptide (VIP)- immunoreactive nerve fibers were observed in the pineals of the rabbit, cat and pig (Uddman et al. 1980). As has been shown in the cat by means of immuno-electron microscopy, VIP-immunoreactive material is confined to large dense-cored vesicles in the intrapineal nerve endings (Møller et al. 1981). VIP-immunoreactive nerve fibers are to a lesser extent also present in the pineal organ of the rat and the Mongolian gerbil. The origin of these nerve fibers has not yet been clarified.

Nerve terminals binding antibodies against substance P were demonstrated in the pineal organs of the Mongolian gerbil (Fig. 13), rat (Fig. 14; cf. Rønnekleiv and Kelley 1984), hedgehog (Fig. 15) and the rhesus monkey (Rønnekleiv et al. 1984). In the rat, these nerve fibers were shown to persist after bilateral ablation of the superior cervical ganglia and, thus, do not represent sympathetic nerve fibers (Rønnekleiv and Kelley 1984). These axons may continue into the pineal organ from a dense network of substance P-immunoreactive nerve fibers innervating the habenular complex and the nucleus of the posterior commissure.

By means of the Falck-Hillarp technique serotonin was localized to the noradrenergic nerve fibers originating from the superior cervical ganglia (Bertler et al. 1964; Nielsen and Møller 1975). This result leads to the conclusion that noradrenergic terminals take up serotonin released from pinealocytes and store it within their terminal formations (Bertler et al. 1964; Bak et al. 1970; Matsuura 1977; Juillard and Collin 1980). Electric stimulation of the preganglionic nerve fibers of the superior cervical ganglia resulted in a simultaneous release of noradrenaline and serotonin (Jaim-Etcheverry and Zieher 1980). However, recent studies provide convincing evidence that serotonin may also act as a neurotransmitter in central pinealopetal nerve fibers. Following bilateral ablation of the superior cervical ganglia in the dog, a considerable number of serotonin-immunoreactive nerve fibers persisted in the pineal stalk. The demonstration of serotonin-immunoreactive nerve fibers penetrating into the pineal stalk from the brain in the rat (Matsuura et al. 1983b), guinea pig (Tramu et al. 1983), Mongolian gerbil (Fig. 18) and hedgehog (Fig. 19) is in good agreement with the results in the dog. The source of these central serotoninergic nerve fibers may be sought in the raphe nuclei, which contain the most prominent accumulation of serotoninergic perikarya of the brain. However, such perikarya are also present in the habenular nucleus (Fig. 17). The immunocytochemical demonstration of central serotoninergic nerve fibers conforms to the results obtained





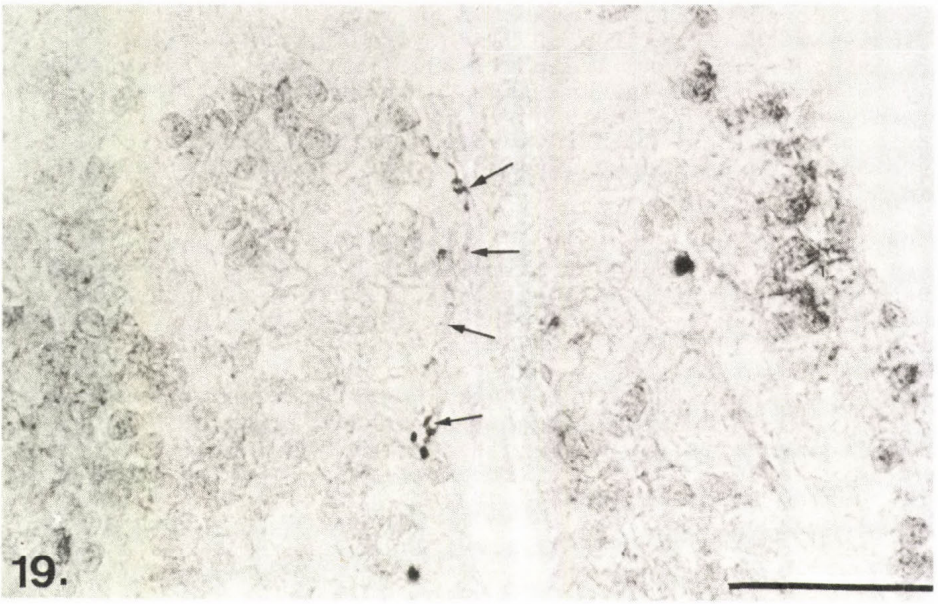


Fig. 17. Frontal section through the habenular complex of the rat containing serotonin-immunoreactive perikarya. Bar indicates 50 µm.
Fig. 18. Sagittal section through the superficial pineal of the Mongolian gerbil. Nerve fibers displaying serotonin-immunoreactivity (arrows). Pinealocytes exhibit a strong immunoreactive stain. Bar indicates 50 µm.
Fig. 19. Frontal section through the pineal of a hibernating hedgehog. Serotonin-immunoreactive nerve fibers (arrows). Pinealocytes contain only moderate amount of immunoreactive material. Bar indicates 50 µm.

after lesions in the habenular nuclei in the rat resulting in degeneration of boutons of the aminergic type (Rønnekleiv and Møller 1979).

FUNCTIONAL ASPECTS OF PEPTIDERGIC PINEALOPETAL PROJECTIONS

The peptidergic terminals are predominantly found in juxtaposition to intrapineal blood vessels; on the other hand, terminals of this type intermingle with the pineal parenchyma. From this topographical arrangement it may be concluded that the neuropeptides are primarily released into the perivascular space. To date, the functional significance of the peptidergic pinealopetal axons is open to discussion. Peptide hormones containing disulfide groups like vasopressin and oxytocin are involved in the inactivation of the pineal N-acetyltransferase under in-vitro conditions (Namboodiri et al. 1981).

Substance P stimulates the activity of the adenylate cyclase in the pineal organ (Duffy et al. 1975). Via this mechanism substance P may modulate the noradrenergic input to the pineal. Noradrenalin by means of an increase in cyclic AMP stimulates the formation of N-acetyltransferase (Klein et al. 1971; Deguchi and Axelrod 1973). Furthermore, substance P may interact in the pineal organ with serotonin released from pinealocytes, sympathetic nerve fibers or central serotoninergic projections. Such interaction between substance P and serotonin has been demonstrated in the dorsal horn (Davies and Roberts 1981).

By means of biochemical in-vitro studies vasoactive intestinal peptide was shown to stimulate the activity of N-acetyltransferase in the pinealocytes of the rat via a non-beta receptor mechanism (Yuwiler 1983). Studies on highly specific binding sites for this peptide in the pineal organ of the rat and the Mongolian gerbil also point toward a direct influence of VIP on pineal function. These investigations were performed on the superficial portion of the pineal glands of rats and Mongolian gerbils rapidly excised after the decapitation of the animals. The binding of radioactively labelled, iodinated VIP showed time-dependent saturation kinetics. The radioactively labelled tracer could be displaced by adding a surplus of non-labelled VIP to the incubation medium (Fig. 20). These results indicate the presence of highly specific binding sites (receptors) for VIP in the pineal of the rat and the Mongolian gerbil. The biochemical data are completed by autoradiographic observations revealing evenly distributed binding of the labelled tracer to the membranes of the pinealocytes in the superficial pineal organ. The binding studies present additional evidence for the functional significance of the peptidergic nerve fibers innervating the mammalian pineal organ. However, further studies are needed to elucidate the precise role of these elements in the regulation of pineal function.

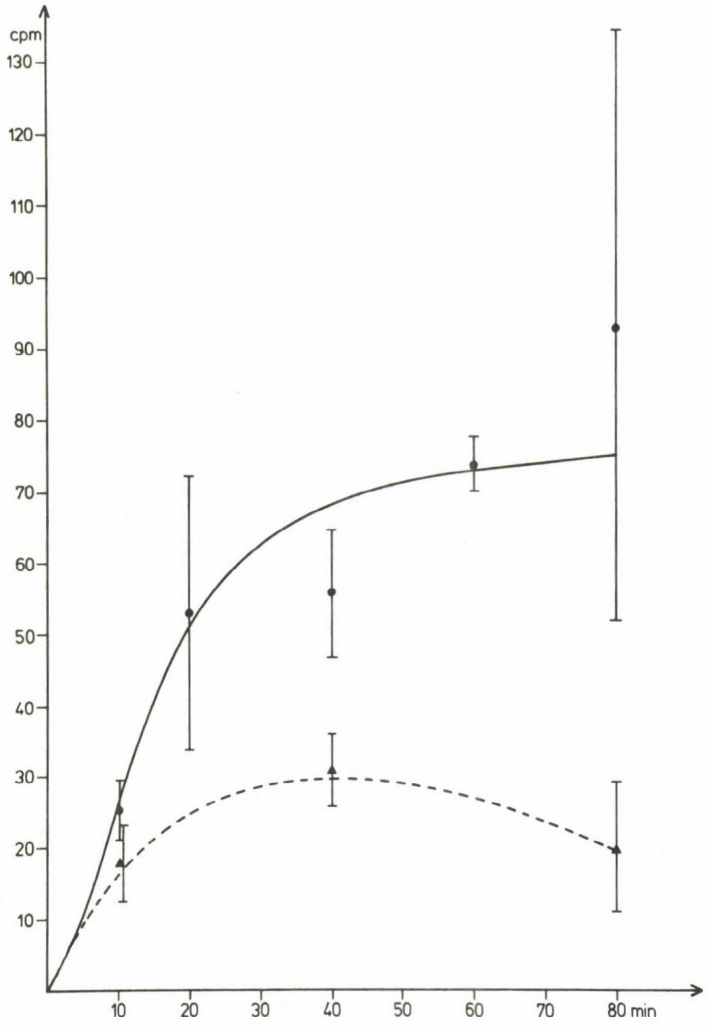
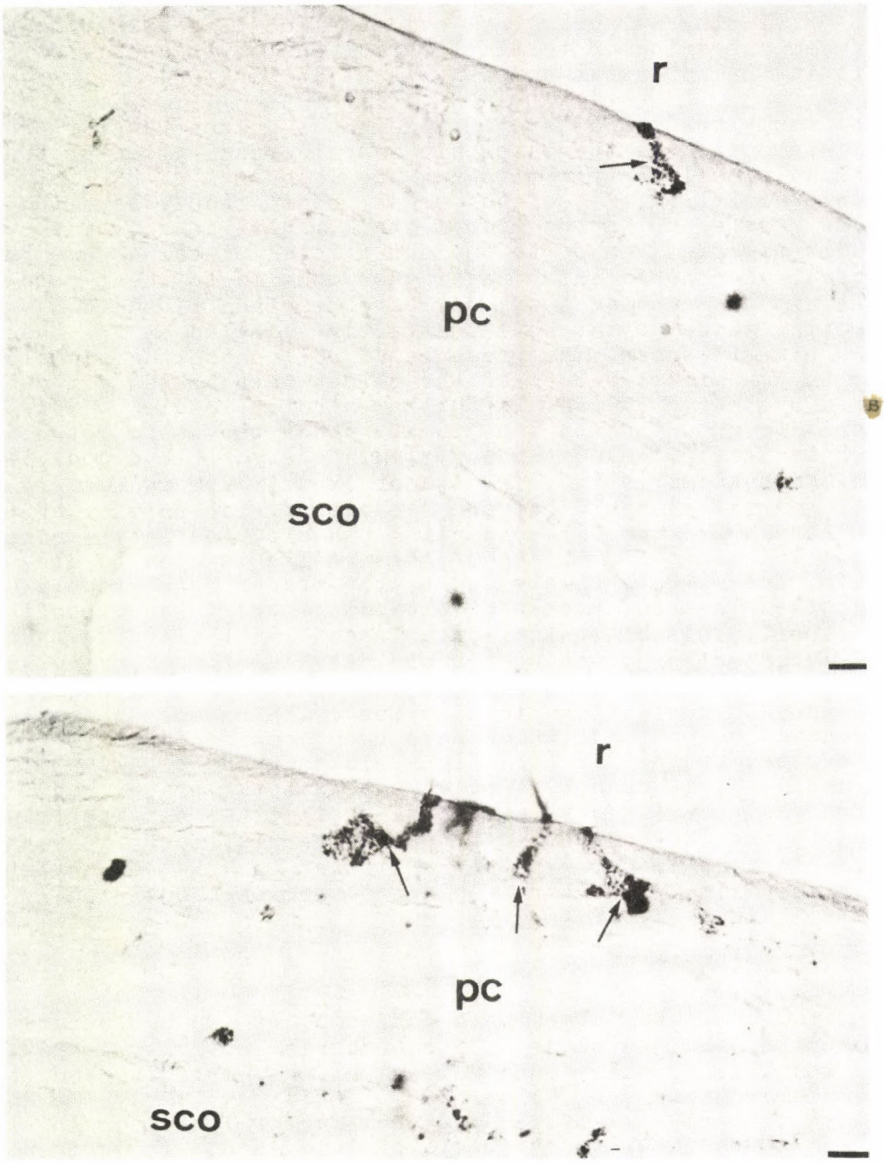


Fig. 20. Time-dependent binding of ^{125}J-VIP to the superficial pineal of the rat. The upper curve (dots) shows the "total" binding of the ligand to the organ. The lower curve (triangles) shows displacement due to adding a surplus of non-labelled ligand to the incubation medium. The curves indicate that binding is saturated after 60 to 80 min. Approximately 75% of the binding can be displaced by adding a surplus of non-labelled ligand. Values are expressed as mean $\pm$ SD. n=24

THE PROBLEM OF CENTRAL PINEALOFUGAL PROJECTIONS

The problem of central pinealofugal nerve fibers must be viewed in context with the function(s) of the mammalian pinealocytes and the presence of nerve cells in the pineal organ of different mammalian species. The endocrine capacity of the mammalian pinealocytes is well documented (cf. Reiter 1982); however, as has been recently pointed out by Quay (1984) the problem of a possible sensory capacity of the mammalian pineal organ is still open to discussion. Mammalian pinealocytes have developed from pineal sensory cells of lower vertebrates (Oksche 1971; Collin 1971; Collin and Oksche 1981). By means of neurophysiological (cf. Dodt 1973; Meissl and Dodt 1981) and morphological (cf. Oksche 1984) methods the latter have been proven to represent photoreceptors. Although to date a direct sensitivity to light has not been demonstrated in the mammalian pineal organ, there are several indications that - at least a specialized population of mammalian pinealocytes - has not lost the essential sensory prerequisites: 1) Pinealocytes of several mammalian species may be endowed with cilia of the 9+0 type (Møller 1976; Vigh-Teichmann and Vigh 1979; McNulty et al. 1980), per se an arrangement typical for receptive elements. 2) Mammalian pinealocytes may contain synaptic ribbons and vesicles (Vollrath 1973; Vigh-Teichmann and Vigh 1979) characteristic of the photoreceptor cells in the pineal complex of lower vertebrates (cf. Vollrath 1981). 3) Rhodopsin kinase, an enzyme of retinal photoreceptor cells has recently also been detected in the pineal organ of the rat (Klein and Somers 1984). 4) Electrophysiological techniques point toward neuronal properties of mammalian pinealocytes (Semm 1983).

In the light of these findings the functional significance of the neurons present in the pineal organ of different mammalian species is in need of reconsideration (Korf and Møller 1984; Quay 1984). The view that all of these intrapineal nerve cells might represent autonomic neurons of the parasympathetic system (Kenny 1965; Romijn 1975) leaves some aspects open. In the ferret and the monkey (David and Herbert 1973; David et al. 1975), clear-cut evidence has been obtained that the intrapineal nerve cells are elements of the central nervous system. Whether they are secondary neurons related to sensory pinealocytes, and thus homologues to the intrapineal nerve cells of lower vertebrates (Wake et al. 1974; Korf 1974,1976), needs further experimental elucidation. This hypothesis, as proposed by David and Herbert (1973) and Vigh-Teichmann and Vigh (1979), might implicate the existence of pinealofugal projections in mammals. Whereas electrophysiological experiments indicate a neural output of the mammalian pineal organ modulating the electrical activity of the habenular nuclei (Semm 1983), pinealofugal nerve fibers have been never demonstrated in a number of previous tracer studies. However, recent experiments facilitating the analysis of the anterograde transport of horseradish peroxidase by the use of the tetramethylbenzidine (TMB) method revealed labelled nerve fibers in the medial habenular nuclei of the golden hamster,



Figs. 21, 22. Frontal sections through the pineal recess (r) at the level of the posterior commissure (pc) of the golden hamster. HRP-labelled cells (arrows). The tracer was injected into the superficial pineal of the animals. Subcommissural organ (sco). Bar indicates 50 µm.

which could not be traced back to perikarya. Thus, one may speculate that they may represent pinealofugal elements. However, convincing morphological evidence for this assumption, i. e. demonstration of terminal formations or branching of the fibers in the habenular complex, is still lacking.

The newly performed experiments in the golden hamster provide information on the intrinsic neural organization of the mammalian pineal organ. Following the injection of HRP into the superficial portion of the organ, retrogradely labelled neurons were found in the pineal stalk. These neurons may transmit signals from or to the superficial pineal organ. Furthermore, when horseradish peroxidase coupled to wheat-germ agglutinin was used as a tracer, cerebrospinal fluid (CSF)-contacting pinealocytes were constantly labelled at the base of the pineal recess. The presence of CSF-contacting pinealocytes in the pineal recess of the golden hamster was demonstrated by Hewing (1978). In further studies, Hewing (1984) was able to show that in the pineal recess the ratio between CSF-contacting pinealocytes and glial cells displays conspicuous seasonal variation. The tracer experiments indicate a functional relationship between the superficial portion of the pineal organ and the CSF-contacting pinealocytes in the pineal recess. The mode of staining of these cells is open to discussion. They might be labelled by the tracer retrogradely transported in their processes extending toward the superficial pineal. This anatomical arrangement might indicate that the CSF-contacting pinealocytes are capable of monitoring signals from the cerebrospinal fluid to the superficial pineal. Thus, the CSF-contacting pinealocytes might closely resemble CSF-contacting neurons, which have been considered as receptors exposed to the CSF (cf. Vigh-Teichmann and Vigh 1983). Alternatively, it cannot be ruled out that the tracer had entered the pineal recess or pineal capillaries and was transported to the CSF-contacting pinealocytes via these pathways. Considering the latter hypothesis it must be stated that only a very limited number of pinealocytes was consistently labelled (Figs. 21, 22).

GENERAL CONSIDERATIONS

The mammalian pineal organ is a component of the neuroendocrine system and, by means of its endocrine activity, capable to modulate or phase-tune several of the circadian and/or circannual rhythms (cf. Reiter 1980, 1981; Quay 1984). To date the most properly investigated pineal factor is melatonin. It has been shown that the synthesis of this indole is influenced by environmental illumination (cf. Reiter 1981). Photoperiodic stimuli can be conveyed to the pineal organ via a polysynaptic chain including the retina, the retinohypothalamic pathway, the suprachiasmatic nucleus and the sympathetic pinealopetal projections originating from the superior cervical ganglia (cf. Moore 1978; Klein 1982). According to our current knowledge, major aspects of the synthetic and functional activities of the pineal organ depend on or are greatly influenced by the sympathetic nerve fibers. Thus, the indole metabolism

of the pineal is under the influence of the sympathetic noradrenergic system (cf. Klein 1982, for review).

As reviewed in this paper, the innervation of the mammalian pineal organ is not restricted to sympathetic nerve fibers. Direct neural connections between the central nervous system and the pineal organ were shown to exist in all mammalian species investigated to date with the use of modern morphological and electrophysiological techniques. Central connections of the mammalian pineal organ must be regarded as an expression of a structural and functional pattern. The immunocytochemical and ultrastructural observations indicate that (i) the cellular elements of the pineal organ are influenced by central nerve fibers by means of a release of their transmitter substances into the perivascular space, or (ii) these fibers may act on single cells (pinealocytes or neurons) via direct apposition to the pinealocytic or neuronal membrane.

The terminal formations of central pinealopetal projections are not evenly distributed throughout the pineal organ. This arrangement might indicate that central projections transmit their signals primarily to specialized subpopulations of pinealocytes. Spreading of these signals to pinealocytes not directly exposed to the central fibers may be facilitated by the presence of gap junctions as demonstrated in the pineal organ of the human fetus (Møller 1976), rat (Taugner et al. 1981) and guinea pig (Huang and Taugner 1983).

The pinealopetal projections of central origin display a rich diversity with respect to the character of the transmitters and their origin. The elucidation of the origin of these elements reveals a pattern common in all rodent species investigated to date. The fibers originate from neurons located in the habenular complex, the hypothalamic paraventricular nucleus and the nucleus of the posterior commissure. Furthermore, optic centers in the diencephalon and mesencephalon project directly to the pineal organ. From a comparative point of view it is noteworthy that the pineal complex of lower vertebrates is also neurally coupled to these centers. However, in these species the projections mainly run in a pinealofugal direction.

The morphological findings on the central pinealopetal projections in mammals conform to electrophysiological results thus indicating that (i) photic stimuli may be transmitted to the pineal organ not only via the sympathetic innervation but also via more direct central routes (Dafny 1980; Semm 1981, 1983); (ii) the mammalian pineal organ is under influence of the olfactory and limbic system; and (iii) the neuroendocrine apparatus of the hypothalamus is involved in the regulation of pineal functions via direct neural pathways (for correlated electrophysiological aspects, see Semm 1983; Reuss et al. 1984). The paraventricular nucleus, an integral component in neuroendocrine and autonomic control mechanisms (Swanson and Sawchenko 1980; for comparative data, see Korf 1984), obviously also plays a role in the regulation of biorhythmic process-

es (cf. Nürnberger and Korf 1981). The direct connections between the paraventricular nucleus and the pineal organ might serve as a neuronal short-loop feed-back from the neuroendocrine apparatus of the hypothalamus, which is considered as a major target area for pineal hormones (cf. Kappers 1978). Furthermore, the paraventricular nucleus apparently represents an important component of the neuronal chain conveying signals from the suprachiasmatic nucleus to the superior cervical ganglia (Klein et al. 1983). The projections related to the olfactory and limbic system may serve the transmission of environmental stimuli differing from those concerned with the photoperiod (Reiter et al. 1971).

It is open to discussion whether the central fibers participate in the regulation of the indole metabolism of the pinealocytes thus interferring with the noradrenergic input to the pinealocyte. On the other hand, central projections may be involved in the control of other pineal functions which to date are not well established. However, there is now an increasing body of evidence that the pineal organ may synthesize and secrete peptides in addition to the well-known indoles (cf. Pévet 1981).

The precise role of direct connections between the central nervous system and the mammalian pineal organ can be elucidated solely by means of future morphological, physiological and biochemical experiments. In these investigations the problem of the intrapineal nerve cells and putative pinealofugal projections should also be considered.

REFERENCES

Bak IJ, Kim JH, Hassler R (1970) Electron microscopic autoradiography for demonstration of pineal serotonin in the rat. Z Zellforsch 105:169-175

Bertler A, Falck B, Owman Ch (1964) Studies on 5-hydroxytryptamine stores in pineal gland and subcommissural organ of the rat. Acta Physiol Scand 63 (Suppl 239):1-18

Björklund A, Owman Ch, West KA (1972) Peripheral sympathetic innervation and serotonin cells in the habenular region of the rat brain. Z Zellforsch 127:510-519

Buijs RM, Pevét P (1980) Vasopressin- and oxytocin-containing fibers in the pineal gland and subcommissural organ of the rat. Cell Tissue Res 205:11-17

Collin JP (1971) Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In: The pineal gland. (Wolstenholme GEW, Knight J, eds). Edinburgh and London: Churchill Livingstone, pp 79-125

Collin JP, Oksche A (1981) Structural and functional relationships in the nonmammalian pineal gland. In: The pineal gland, Vol. 1. Anatomy and biochemistry. (Reiter RJ, ed). Boca Raton: CRC Press, pp 27-67

Dafny N (1980) Photic input to rat pineal gland conveyed by both sympathetic and central afferents. J Neural Transm 48: 203-208

Dafny N (1983) Evidence that the rat pineal has neuronal connections via the pineal stalk. Exp Neurol 79: 858-861
David GFX, Herbert J (1973) Experimental evidence for a synaptic connection between habenula and pineal ganglion in the ferret. Brain Res 64:327-343
David GFX, Herbert J, Wright GDS (1973) The ultrastructure of the pineal ganglion in the ferret. J Anat 115:79-97
David GFX, Umberkoman B, Kumar K, Anand Kumar TC (1975) Neuroendocrine significance of the pineal. In: Brain-endocrine interaction II (Knigge KM, Scott DE, Kobayashi H, Ishii S, eds). Basel: Karger, pp. 365-375
Davies JE, Roberts MHT (1981) 5-hydroxytryptamine reduces substance P responses on dorsal horn interneurons: a possible interaction of neurotransmitters. Brain Res 217:399-404
Deguchi T, Axelrod J (1973) Superinduction of serotonin N-acetyltransferase and supersensitivity of adenyl cyclase to catecholamines in denervated pineal gland. Mol Pharmacol 9:612-618
Ekström P (1984) Central neural connections of the pineal organ and retina in the teleost Gasterosteus aculeatus L. J Comp Neurol 226: 321-335
Ekström P, van Veen Th (1983) Central connections of the pineal organ of the three-spined stickleback, Gasterosteus aculeatus L. (Teleostei). Cell Tissue Res 232:141-155
Eldred WD, Finger TE, Nolte J (1980) Central projections of the frontal organ of Rana pipiens, as demonstrated by the anterograde transport of horseradish peroxidase. Cell Tissue Res 211:215-222
Guerillot C, Pfister A, Müller J, Da Lage C (1982) Recherche de l'origine des fibres nerveuses extraorthosympathiques innervant l'epiphyse du rat (etude du transport retrograde de la peroxydase de raifort). Reprod Nutr Dev 22:371-378
Hewing M (1976) Die postnatale Entwicklung der Epiphysis cerebri beim Goldhamster. Verh Anat Ges 70: 85-92
Hewing M (1978) A liquor contacting area in the pineal recess of the golden hamster (Mesocricetus auratus). Anat Embryol 153:295-304
Hewing M (1984) Seasonal variations in the cerebrospinal fluid contacting area of the pineal gland in the golden hamster Mesocricetus auratus). Anat Embryol 169:91-96
Hosaka T, Nakai A, Kushima S (1957) Innervation of the pineal body and the supracommissural and subcommissural organs in the Japanese monkey. Bull Tokyo Med Dent Univ 4:365-378
Huang SK, Taugner R (1983) Gap junctions between guinea pig pinealocytes. Cell Tissue Res
Jaim-Etcheverry G, Zieher LM (1980) Stimulation-depletion of serotonin and noradrenaline from vesicles of sympathetic nerve fibers in the pineal gland of the rat. Cell Tissue Res 207:13-20
Juillard MT, Collin JP (1980) Pools of serotonin in the pineal gland of the mouse: the mammalian pinealocyte as a component of the diffuse neuroendocrine system. Cell Tissue Res 213: 273-291
Kappers JA (1960) The development, topographical relations, and innervation of the epiphysis cerebri in the albino rat. Z Zellforsch 52:163-215

Kappers JA (1965) Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Prog Brain Res 10:87-153

Kappers JA (1976) The mammalian pineal gland, a survey. Acta neurochir (Wien) 34:109-149

Kenny GCT (1965) The innervation of the mammalian pineal body. (A comparative study). Proc Aust Assoc Neurol 3:133-140

Klein DC (1982) Melatonin rhythm generating system. Developmental aspects. Basel: Karger

Klein DC, Somers R (1984) Presence of rhodopsin kinase in the rat pineal gland. J Steroid Biochem 20, 6B: 1455

Klein DC, Weller JL, Moore RY (1971) Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzyme A N-acetyltransferase activity. Proc Natl Acad Sci 68: 3107-3110

Klein DC, Smoot R, Weller JL, Higa S, Markey SP, Creed GJ, Jacobowitz DM (1983) Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic-spinal cord circuit in the melatonin rhythm generating system. Brain Res Bull 10:647-652

Korf HW (1974) Acetylcholinesterase-positive neurons in the pineal and parapineal organs of the rainbow trout, Salmo gairdneri (with special reference to the pineal tract). Cell Tissue Res 155:475-489

Korf HW (1976) Histological, histochemical and electron microscopical studies on the nervous apparatus of the pineal organ of the tiger salamander, Ambystoma tigrinum. Cell Tissue Res. 174:475-497

Korf HW (1984) The neuronal organization of the avian paraventricular nucleus: intrinsic, afferent and efferent connections. J Exp Zool (in press)

Korf HW, Wagner U (1980) Evidence for a nervous connection between the brain and the pineal organ in the guinea pig. Cell Tissue Res 209:505-510

Korf HW, Wagner U (1981) Nervous connections of the parietal eye in adult Lacerta s. sicula Rafinesque as demonstrated by anterograde and retrograde transport of horseradish peroxidase. Cell Tissue Res 219:567-584

Korf HW, Møller M (1983) Zentrale Innervation der Epiphyse bei verschiedenen Säugetierarten. Verh Anat Ges 77:

Korf HW, Møller M (1984) The innervation of the mammalian pineal gland with special reference to central pinealopetal projections. In: Pineal research reviews (Reiter RJ, ed) Vol. 2. New York: Alan Liss, pp 41-86

Korf HW, Vigh-Teichmann I (1984) Sensory and central nervous elements in the avian pineal organ. Ophthalmic Res 16: 96-101

Korf HW, Zimmerman NH, Oksche A (1982) Intrinsic neurons and neural connections of the pineal organ of the house sparrow, Passer domesticus, as revealed by anterograde and retrograde transport of horseradish peroxidase. Cell Tissue Res 222: 243-260

Le Gros Clark WE (1940) The nervous and vascular relations of the pineal gland. J Anat 74:470-494

Lin HS, Hwang HB, Tseng CY (1975) Fine structural changes in the hamster pineal gland after blinding and superior

cervical ganglionectomy. Cell Tissue Res 158:285-299
Lues G (1971) Die Feinstruktur der Zirbeldrüse normaler, trächtiger und experimentell beeinflußter Meerschweinchen. Z Zellforsch 114:38-60
Matsuura T (1977) The influence of chemical and surgical sympathectomy on the rat pineal body. J Kyoto Pref Univ Med 86:493-507
Matsuura T, Sano Y (1983) Distribution of monoamine-containing nerve fibers in the pineal organ of untreated and sympathectomized dogs. Cell Tissue Res 234:519-531
Matsuura T, Kawata M, Yamada H, Kojima M, Sano Y (1983a) Immunohistochemical studies on the peptidergic nerve fibers in the pineal organ of the dog. Arch Histol Jpn 46: 373-379
Matsuura T, Takeuchi Y, Sano Y (1983b) Immunohistochemical and electron microscopical studies on serotonin containing nerve fibers in the pineal organ of the rat. Biomed Res 4: 261-270
McNulty JA, Hazlett JC (1980) The pineal region in the opossum Didelphis virginiana. I. Ultrastructural observations. Cell Tissue Res 207:109-121
Meissl H, Dodt E (1981) Comparative physiology of pineal photoreceptor organs. Develop Endocrinol 14:61-80
Møller M (1976) The ultrastructure of the human fetal pineal gland. II. Innervation and cell junctions. Cell Tissue Res 169:7-21
Møller M (1978) Presence of a pineal nerve (nervus pinealis) in the human fetus: a light and electron microscopical study of the innervation of the pineal gland. Brain Res 154: 1-12
Møller M (1979) Presence of a pineal nerve (nervus pinealis) in fetal mammals. Prog Brain Res 52:103-106
Møller M, Korf HW (1983a) Central innervation of the pineal organ of the Mongolian gerbil. A histochemical and lesion study. Cell Tissue Res 230:259-272
Møller M, Korf HW (1983b) The origin of central pinealopetal nerve fibers in the Mongolian gerbil as demonstrated by the retrograde transport of horseradish peroxidase. Cell Tissue Res 230:273-287
Møller M, Møllgard K, Kimble JE (1975) Presence of a pineal nerve in sheep and rabbit fetuses. Cell Tissue Res 158: 451-459
Møller M, Fahrenkrug J, Ottesen B (1981) The presence of vasoactive intestinal peptide (VIP) in nerve fibers connecting the brain and the pineal organ of the cat. Neurosci Lett (Suppl) 7:441
Møllgard K, Møller M (1973) On the innervation of the human fetal pineal gland. Brain Res. 52:428-432
Namboodiri MAA, Favilla JT, Klein DC (1981) Pineal N-acetyltransferase is inactivated by disulfide-containing peptides: Insulin is the most potent. Science 213:571-573
Nielsen JT, Møller M (1975) Nervous connections between the brain and the pineal in the cat (Felis catus) and the monkey (Cercopithecus aethiops). Cell Tissue Res 161: 293-301
Nielsen JT, Møller M (1978) Innervation of the pineal gland in the Mongolian gerbil (Meriones unguiculatus): a

fluorescence microscopical study. Cell Tissue Res 187: 235-250
Nürnberger F, Korf HW (1981) Oxytocin- and vasopressin-immunoreactive nerve fibers in the pineal gland of the hedgehog, Erinaceus europaeus L. Cell Tissue Res 220:87-97
Oksche A (1971) Sensory and glandular elements of the pineal organ. In: The pineal gland (Wolstenholme GEW, Knight J, eds). Edinburgh and London: Churchill-Livingstone, pp 127-146
Oksche A (1983) Aspects of evolution of the pineal organ. In: The pineal gland and its endocrine role. (Axelrod J, Fraschini F, Velo GP, eds). New York: Plenum Press, pp 15-35
Oksche A (1984) Evolution of the pineal complex:correlation of structure and function. Ophthalmic Res 16:88-95
Pfister A, Müller J, Leffray P, Guerillot C, Vendrely E, Da Lage C (1978) Investigation on a possible extraorthosympathetic innervation of the pineal in rat and hamster. J Neural Transm (Suppl) 13:390-391
Quay WB (1984) Pineal function in mammals: a reconsideration. In: Pineal research reviews (Reiter RJ, ed). New York: Alan Liss, pp 87-112
Reiter RJ (1980) The pineal and its hormones in the control of reproduction in mammals. Endocrinol Rev 1:109-131
Reiter RJ (ed) (1981) The pineal gland, Vol. 1. Boca Raton: CRC Press
Reiter RJ (ed) (1982) The pineal gland, Vol. 2. Boca Raton: CRC Press
Reiter RJ, Sorrentino S, Ralph CL, Lynch KJ, Mull D, Jarrow E (1971) Some endocrine effects of blinding and anosmia in adult male rats with observations on pineal melatonin. Endocrinol 85:895-900
Reuss St, Semm P, Vollrath L (1984) Electrophysiological investigations on the central innervation of the rat and guinea pig pineal gland. J Neural Transm (in press)
Romijn HJ (1973) Structure and innervation of the pineal gland of the rabbit, Oryctolagus cuniculus (L.). A light microscopic investigation. Z Zellforsch 139: 473-485
Romijn HJ (1975) Structure and innervation of the pineal gland of the rabbit, Oryctolagus cuniculus (L.). III. An electron microscopic investigation of the innervation. Cell Tissue Res 157:25-51
Rønnekleiv O, Møller M (1979) Brain-pineal nervous connections in the rat: an ultrastructural study following habenular lesion. Exp Brain Res 37:551-562
Rønnekleiv OK,Kelley MJ (1984) Distribution of substance P neurons in the epithalamus of the rat: an immunohistochemical investigation. Pineal Res (in press)
Rønnekleiv OK, Hess DL, Resko JA, Naylor BR (1984) Immunohistochemical studies of developing peptidergic and noradrenergic innervation of the prosimian (Galago crassicaudatus) and the macaque (rhesus) pineal. Anat Rec 208:151
Scharenberg K, Liss L (1965) The histologic structure of the human pineal body. Prog Brain Res 10:193-217
Schneider T, Semm P, Vollrath L (1981) Ultrastructural observations on the central innervation of the guinea-pig pineal gland. Cell Tissue Res 220:41-49

Semm P (1981) Electrophysiological aspects of the mammalian pineal gland. Develop Endocrinol 14: 81-96
Semm P (1983) Neurobiological investigations of the pineal gland and its hormone melatonin. In: The pineal gland and its endocrine role (Axelrod J, Fraschini F, Velo GP, eds). New York: Plenum Press, pp 437-465
Taugner R, Schiller A, Rix E (1981) Gap junctions between pinealocytes. A freeze-fracture study of the pineal gland in rats. Cell Tissue Res 218:303-314
Tramu G, Pillez A, Leonardielli J (1983) Serotonin axons of the ependyma and circumventricular organs in the forebrain of the guinea pig. Cell Tissue Res 228:297-311
Uddman, R, Alumets J, Håkanson R, Lorén I, Sundler F (1980) Vasoactive intestinal peptide (VIP) occurs in nerves of the pineal gland. Experientia 36:1119-1120
Ueck M (1979) The innervation of the vertebrate pineal. Prog Brain Res 52:44-87
Vigh-Teichmann I, Vigh B (1979) Comparison of the epithalamic, hypothalamic and spinal neurosecretory terminals. Acta Biol Acad Sci Hung 30:1-39
Vigh-Teichmann I, Vigh B (1983) The system of cerebrospinal fluid-contacting neurons. Arch Histol Jpn 46:427-468
Vollrath L (1973) Synaptic ribbons of a mammalian pineal gland. Circadian changes. Z Zellforsch 145:171-183
Vollrath L (1981) The pineal organ. In: Handbuch der mikroskopischen Anatomie des Menschen. Bd VI/7 (Oksche A, Vollrath L, eds). Berlin: Springer, pp 1-665
Wake K, Ueck M, Oksche A (1974) Acetylcholinesterase-containing nerve cells in the pineal complex and subcommissural area of the frogs, Rana ridibunda and Rana esculenta. Cell Tissue Res 154:423-442
Weindl A, Sofroniew MV (1982) Peptide neurohormones and circumventricular organs. In: Frontiers in hormone research (van Wimersma Greidanus TjB, Rodríguez EM, eds). Basel: Karger, pp 88-104
Wiklund L (1974) Development of serotonin-containing cells and the sympathetic innervation of the habenular region in the brain. A fluorescence histochemical study. Cell Tissue Res 155:231-243
Yulis CR, Rodríguez EM (1982) Neurophysin pathways in the normal and hypophysectomized rat. Cell Tissue Res 227:93-112
Yuwiler A (1983) Vasoactive intestinal peptide stimulation of pineal serotonin-N-acetyltransferase activity: general characteristics. J Neurochem 41:146-153
Zweig M, Snyder SH, Axelrod J (1966) Evidence for a nonretinal pathway of light to pineal gland in newborn rats. Proc Natl Acad Sci USA 56:515-520
Zuo ZR, Schultz RL, Whitter EF, Vollrath L (1984) The ultrastructure of the nerve fibers and pinealocytes in the rat pineal stalk. Pineal Res (in press)

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CSF-CONTACTING NEURONS AND PINEALOCYTES

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The comparative study of cerebrospinal fluid (CSF)-contacting neurons and pinealocytes has been rather helpful in elucidating details of the organization of both the pineal complex and the CSF-contacting neuronal system. In the following, we try to summarize some interesting questions of this work.

WHAT ARE CSF-CONTACTING NEURONS?

CSF-contacting neurons send one of their processes -ciliated dendritic terminals, or axons - into the CSF, or their perikarya are entirely located within the lumen of the brain ventricles of vertebrate species (Vigh and Vigh-Teichmann 1973, Vigh-Teichmann and Vigh 1974, 1983, Vigh-Teichmann et al. 1980a, 1981). Most of the CSF-contacting nerve cells were found in the periventricular gray of the hypothalamus and around the central canal of the spinal cord (Vigh et al. 1977, 1979, 1980a,b, 1983a, Vigh and Vigh-Teichmann 1971, 1973), in the lateral and the 4th ventricles (lit., see Leonhardt 1980). The peculiar location of the neuronal elements suggests that their activity may be connected in some manner with the CSF. In addition, some of the CSF-contacting neurons may serve as integrative elements between the various CSF-contacting neuronal areas and the central nervous system (for review, see Vigh-Teichmann and Vigh 1983).

Similarities in structure often suggest similarities in function. Cytology and polarization of the CSF-contacting neurons and their morphological similarity with known receptors offer some working hypotheses in elucidating their role. Presumably, the CSF-contacting neurons of the brain and spinal cord represent the morphological basis for mechano-, thermo-, chemo- and so-called deep encephalic photoreceptors supposed to occur in the ventricular wall by physiological experiments (lit., see Vigh-Teichmann et al. 1976, Hartwig and Oksche 1982, Underwood and Groos 1982).

Are some pineal and retinal cells CSF-contacting neurons?

The CSF-contacting neuronal system also includes the photosensory cells of the pineal complex and retina and further some of the bipolar neurons of the retina that form Landolt's clubs (Landolt 1871) in the "photoreceptor space" between the pigment epithelium and the photosensory cells. All these cells project ciliated dendrites either into the pineal lumen or the retinal photoreceptor space, i.e., into CSF cavities derived during embryonic development from the epithalamic and hypothalamic portions of the diencephalic third ventricle. The photoreceptor cells and the periventricular CSF-contacting neurons exhibit common characteristics by their peculiar orientation around the CSF space and the polarization of their perikarya (Vigh et al. 1975). Perhaps, they alltogether represent a system of central receptor cells of the brain.

Data on the ultrastructural and immunocytochemical properties of the CSF-contacting neurons, pineal photoreceptors and retinal Landolt's clubs of bipolars are still rather incomplete. Further, morphological evidence on the presence of extraretinal deep encephalic photoreceptors is still lacking. Therefore, in the present study we summarize our comparative light and electron microscopic results on the periventricular hypothalamic, spinal and retinal CSF-contacting neurons as well as on retinal and pineal photoreceptors.

MATERIALS AND METHODS

Common light and electron microscopy was performed in all vertebrate classes from the Lancelet and cyclostomes to mammals. Routine staining techniques included chrome alum gallocyanin after previous oxidation with $KMnO_4$-sulfuric acid, toluidine blue-azure II, acetylcholinesterase reaction and silver impregnations according to Golgi and Golgi-Kopsch. Further, immunocytochemical studies were performed by means of polyclonal antibodies raised against somatostatin, 5-hydroxytryptamine (5-HT, serotonin), neurophysins and bovine (rhod)opsin. In addition, the hybridoma supernatants A_1 and D_6 were applied containing antibodies against iod-opsin-containing photoreceptor membranes of chicken retina (Szél et al. 1984). The binding of the antibodies to the antigenic sites was visualized with the light microscopic peroxidase-antiperoxidase (PAP), avidin-biotinperoxidase complex (ABC) techniques followed by diaminobenzidine-H_2O_2, and with the immuno-electron-microscopic protein A-colloidal gold method (for technical details, see Vigh-Teichmann et al. 1970, 1980b, 1982, 1983, 1984a,b, Vigh and Vigh-Teichmann 1981, Vigh et al. 1982, 1983b, 1984, Van Veen et al. 1984). Scales on the pictures are micrometers.

THE PERIVENTRICULAR CSF-CONTACTING NEURONS

The highest number of perikarya of CSF-contacting nerve cells can be found periventricularly from the lancelet to reptiles, while there are less in birds and mammals. Most of the CSF-contacting neurons are located in the hypothalamus, e.g. in the parvocellular preoptic area, the magnocellular preoptic and paraventricular nuclei, the anterior and posterior periventricular nuclei, the paraventricular organ, the vascular sac, the nucleus lateralis tuberis and the infundibular nuclei, etc.). A considerable number of CSF-contacting neurons occur in the gray around the central canal of the oblongate medulla, spinal cord and terminal filum (for lit., see Vigh-Teichmann and Vigh 1983).

Hypothalamic CSF-contacting neurons

The CSF-contacting neurons of the periventricular gray of the hypothalamus are characterized by ciliated CSF-contacting dendrite terminals being often of club- or bulb-shape (Fig.1) and resembling those of known sensory cells. The dendrite terminals ("inner segments", Vigh et al. 1975) of the CSF-contacting neurons of the hypothalamus are endowed with 9x2+0 cilia (corresponding to outer segments) supplied with basal and accessory basal bodies, striated rootlet fiber, further with elements of the endoplasmic reticulum, accumulations of mitochondria and a varying number of granulated vesicles. The 9x2+0 cilia are of a modified type: they are shorter than the kinocilia of the ependymal cells and may exhibit a spiral-like appearance of their ciliary shaft (Vigh-Teichmann et al. 1979). The ciliated CSF-contacting dendrites mainly resemble chemoreceptors, or developing/regressing photoreceptors.

Spinal CSF-contacting neurons

Most of the CSF-contacting dendrites of the CSF-contacting neurons of the spinal cord exhibit a varying number of stereocilia and a solitary kinocilium (type 9x2+2). The stereocilia are filled with parallel microfilaments which project into the distal tapering end of the stereocilia and into the cytoplasm of the dendrite terminals (Vigh et al. 1977). Stereociliated CSF-contacting neurons can be found in all vertebrate classes. The morphological similarity of the stereociliated CSF-contacting dendrites to those of known mechanoreceptors suggests a mechanoreceptive function although a chemoreceptive activity has also to be taken into consideration since stereocilia also occur on chemoreceptors. At any case, recent in-vitro experiments (fictive locomotion) on spinal cord-notocord preparations of the lamprey provided direct evidence that after transection of dorsal and ventral roots there were mechanosensitive neurons intrinsic to the spinal cord (Grillner et al. 1982). It may be suggested that these mechanoreceptive nerve cells correspond to at least some of the stereociliated CSF-contacting neurons.

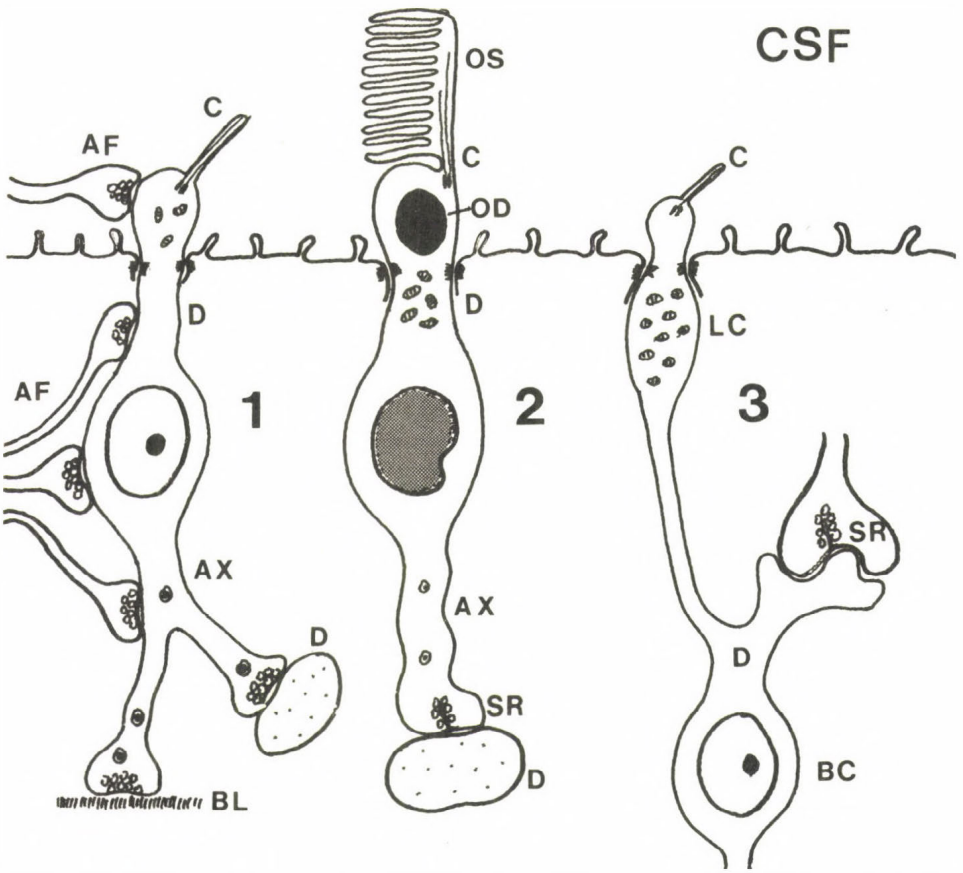


Fig. 1. Scheme comparing CSF-contacting neurons (1), photoreceptors (2) and bipolars of the retina forming Landolt's clubs (3). AF afferents, AX axons, BC bipolar cell of retina, BL basal lamina, C cilia of type 9x2+0, CSF space of the cerebrospinal fluid, D dendrites, LC Landolt's clubs, OD oil droplet of inner segment, OS outer segment of cone-like photoreceptor, SR axon terminals containing synaptic ribbons.

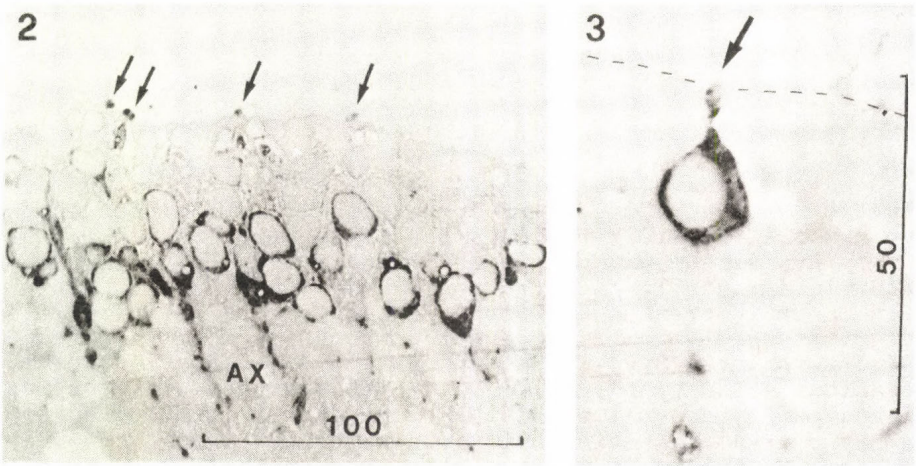
Perikarya of CSF-contacting neurons

The perikarya of both hypothalamic and spinal CSF-contacting neurons are supplied with various types of axo-somatic synapses, those of the peptidergic type included, further with endoplasmic reticulum, Golgi apparatus, mitochondria, granulated vesicles of various sizes, and solitary, double or multiple cilia of type 9x2+0 (lit, see Vigh-Teichmann et al. 1976, 1980, Vigh-Teichmann and Vigh 1979). Such cilia also occur on common nerve cells. We think the cilia resemble those of primitive chemoreceptors and may serve for the detection of

changes of the immediate intercellular environment (ion composition and/or concentration of the intercellular fluid?) of CSF-contacting and non-CSF-contacting nerve cells and their supporting glial cells.

Axons of CSF-contacting neurons

The perikarya or the main ependymofugal dendrite of the periventricular neurons give rise to axons as shown by silver impregnations (lit., see Vigh and Vigh-Teichmann 1973, Vigh-Teichmann and Vigh 1983). Very often, the impregnated axons were only traced over short distances into the periventricular gray. In lower vertebrates, we could follow the main basal processes of CSF-contacting neurons by means of chrome alum gallocyanin staining, acetylcholinesterase reaction, neurophysin or somatostatin immunoreactions into the preoptic-neuro-



Figs 2 and 3. Neurophysin-immunoreactive neurons of the magnocellular preoptic nucleus of Triturus vulgaris (2) and Rana esculenta (3). Arrows immunoreactive CSF-contacting dendrite terminals. The neurosecretory axons (AX) pass into the preoptic-neurohypophyseal tract. Dotted line lining of the 3rd ventricle. Semithin sections, PAP reaction.

hypophyseal tract, the tractus sacci vasculosi or into a somatostatin-immunoreactive fiber plexus, respectively. In teleosts, the latter surrounds the 3rd ventricle in the postchiasmatic hypothalamus and extends to the anterior neurohypophysis (Vigh-Teichmann et al. 1983c). In contrast to the axons of retinal and pineal photoreceptors as well as retinal bipolar neurons the axons of apparent CSF-contacting neurons appear to be devoid of synaptic ribbons.

Bioactive substances in CSF-contacting neurons

The presence and formation of some kind of granulated ve-

sicles do not allow to draw far-reaching conclusions as to the nature of their content. At present, a large number of bioactive substances has been demonstrated in hypothalamic CSF-contacting neurons by immunocytochemistry (lit., see Vigh-Teichmann and Vigh 1983). Thus, according to their immunocytochemical properties they may show differences, a great diversity (Figs 2,3,4,5,6).

Our studies with antibodies raised against somatostatin show that somatostatin-immunoreactive neurons are present in the periventricular area of the hypothalamus of different vertebrate classes. We found somatostatin-positive CSF-contacting neurons in the most caudal part of the infundibular nucleus of Xenopus laevis tadpoles of stage 60 (Blähser et al. 1982), in the anterior periventricular nucleus of the eel, the rainbow trout and the European minnow (Vigh-Teichmann et al. 1983c) and in the paraventricular nucleus of the turtle, Emys orbicularis (Vigh-Teichmann and Vigh 1983). In addition, somatostatin immunoreactive neurons were observed in the entopeduncular, preoptic and dorsolateral thalamic nuclei, the pretectal area and ventrolateral tegmentum of the teleosts studied; these regions and the periventricular nucleus are known to receive afferents from the optic and pineal photoreceptive systems (lit., see Vigh-Teichmann et al. 1983c).

Somatostatin is an inhibiting peptide. Therefore, the somatostatin-immunoreactive CSF-contacting neurons appear to be involved via their afferents in the inhibitive integration of photic information and via their ciliated CSF-contacting dendrites in a possible chemoreceptive activity (Fig. 1). (It would be of interest to know the glucose levels of the CSF during metamorphosis of Xenopus and other species, when feeding cessates, because hypoglycemia increases somatostatin secretion specifically (Lengyel et al. 1984). The somatostatin-positive CSF-contacting neurons might then take part in (photo)neuroendocrine regulation via their neurosecretory axons to neurohemal areas, i.e., the median eminence/anterior neurohypophysis for the regulation of growth hormone release from the adenohypophysis and via their possible transmitter axon collaterals to various brain regions.

Previous studies with induced monoamine fluorescence demonstrated CSF-contacting neurons in the parvocellular preoptic area of amphibians, of some fish and in the paraventricular organ of submammalian vertebrates (lit., see Vigh and Vigh-Teichmann 1973, Vigh-Teichmann and Vigh 1983). Immunocytochemistry now allows to distinguish more easily the types of monoamine-containing cells. In the paraventricular organ, serotonin-containing CSF-contacting neurons were demonstrated immunocytochemically in Cyprinus carpio, Rana catesbeiana, the fowl and Clemnys japonica (Sano et al. 1983). In collaboration with Dr. Theo van Veen and his team (Zoology, Lund) we found 5-hydroxytryptamine-immunoreactive CSF-contacting neurons in the paraventricular organ of Myxine glutinosa, Anguilla anguilla, Phoxinus phoxinus, Gasterosteus aculeatus and Rana esculenta (Figs 4-6). In the hagfish, the serotonin-immunoreactive CSF-contacting nerve cells were observed around the infundibu-

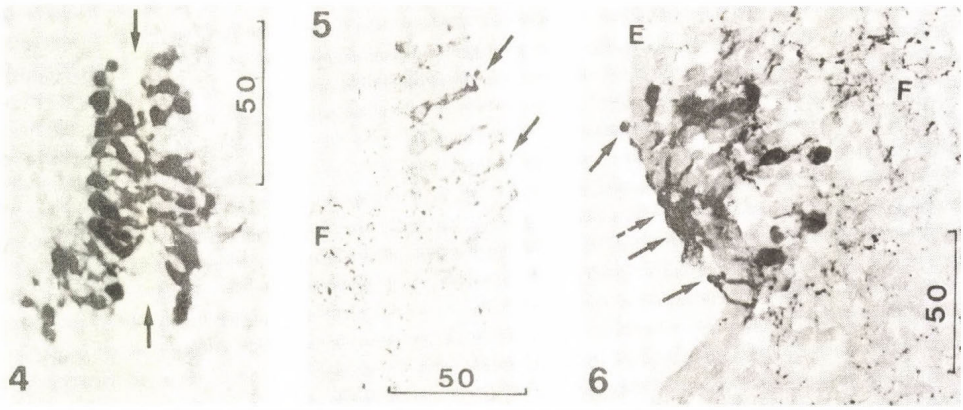


Fig. 4. Serotonin-immunoreactive CSF-contacting neurons of the paraventricular organ of the stickleback, Gasterosteus aculeatus, at about hatching (stage 144hrs). Arrows 3rd ventricle, cryostate section, PAP reaction. (Courtesy of Dr. Theo van Veen of the Department of Zoology, Lund, unpublished photograph).

Fig. 5. Serotonin-immunoreactive CSF-contacting neurons of the paraventricular organ of the European minnow, Phoxinus phoxinus. Arrows CSF-contacting dendrite terminals, F immunoreactive nerve fibers. Semithin section, ABC method.

Fig. 6. Serotonin-immunoreactive CSF-contacting neurons of the paraventricular organ in the frog, Rana esculenta. Arrows CSF-contacting dendrite terminals, E ependyma, F immunoreactive nerve fibers of the neuropil. Cryostate section, PAP method.

lar recess and its lateral extensions, a region that appears to correspond to the paraventricular organ of more differentiated lower vertebrates. In addition, Myxine glutinosa exhibited CSF-contacting neurons in the oblongate medulla and spinal cord (Vigh-Teichmann et al. 1984b).

Since serotonin immunoreactivity also occurs in photoreceptors of the pineal organ (van Veen et al. 1984) it is of interest whether there are also other immunocytochemical similarities between certain CSF-contacting neurons and pinealocytes. In contrast to retinal and pineal photoreceptors, the CSF-contacting ciliated dendrites of the hypothalamus and spinal cord did not exhibit immunoreaction with an antiserum raised against the opsin of the photopigment rhodopsin of the cattle retina (Vigh-Teichmann et al. 1980b). Apparently, the majority of CSF-contacting neurons is not identical with the so-called extraretinal deep encephalic photoreceptors. A final conclusion on the presence of the receptor peptide opsin in CSF-contacting neurons can, however, only be drawn after having performed tests with a broad scale of antibodies raised against different kinds of opsins.

THE PINEAL PHOTORECEPTORS

Cytologically, the photosensory cells of the pineal complex of lower vertebrates are endowed - similarly to the CSF-contacting neurons - with dendrites penetrating as inner segments into the photoreceptor space of the pineal organ, further of the parapineal, frontal and parietal organs. These inner segments exhibit 9x2+0 cilia, the outer segments. Those of the photosensory cells from amphioxus to reptiles possess regular photoreceptor membrane specializations as found in rods and cones of the retina (Fig. 1). In the pineal organ, the outer segments resemble the retinal cones morphologically (lit., see Vollrath 1981).

The pinealocytes of birds and mammals were generally considered to take part in neuroendocrine activity and to have lost the photoreceptive ability of their predecessors, a phenomenon thought to be expressed by the "degenerated", modified structure of the pineal outer segments of birds and the simple 9x2+0 appearance of those of mammalian pinealocytes (lit., see Vigh-Teichmann and Vigh 1979, Vollrath 1981).

Our earlier comparative ultrastructural studies revealed that also the pinealocytes of birds (Gallus domesticus, Columba livia, Melopsittacus undulatus, Serinus canaria, Taeniopyga punctata) are endowed with regular photoreceptor outer segments resembling those of the developing chicken retina and the eye of the hagfish (Vigh et al. 1975, 1982, Vigh-Teichmann et al. 1984). These outer segments exhibit a modified circular arrangement of the photoreceptor membranes around an internal floccular matrix of the cilium and its 9 pairs of tubules. Further, in avian species, a synaptic neuropil was found including synaptic contacts between ribbon-containing axons of pinealocytes and dendrites of intrinsic pineal neurons (Vigh et al. 1975).

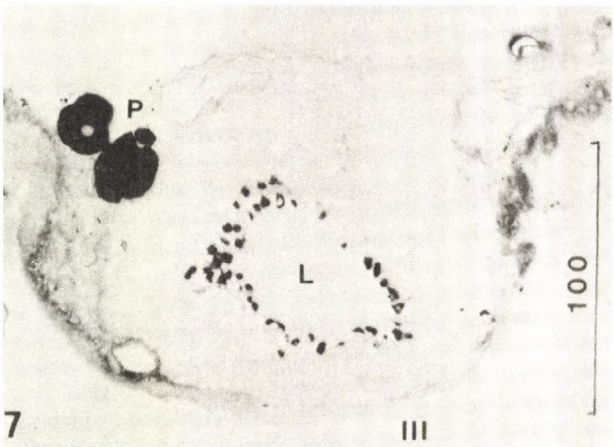
The 9x2+0 cilia of the mammalian pinealocytes contain likewise a floccular material in their interior and they have slight thickenings of their ciliary shaft like those of developing photoreceptors. In insectivorous species, the cilia are insulated by a glial sheet. Typical photoreceptor membranes are, however, absent on the cilia at least in the opossum, the hedgehog, the bat, the rabbit, the rat and the cat (Vigh and Vigh-Teichmann 1981). In the latter species, perikarya and dendrites of intrinsic neurons received ribbon-containing synapses from axons of pinealocytes. Since during evolution incorporation of photopigments first appeared in cell membranes and ciliary or rhabdomic photoreceptor membranes developed later, it has to be clarified whether the avian and mammalian pinealocytes elaborate some kind of photopigment and thereby still possess the capacity to detect light.

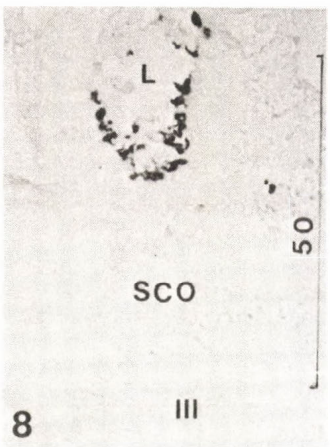
The perikarya of the photoreceptors of the pineal organ elaborate some kind of bioactive material in agranular (fish and amphibians) and granular form (reptiles to birds, mammals).

By means of induced monoamine fluorescence and immunoreaction 5-hydroxytryptamine or its precursor 5-hydroxytryptophan were demonstrated in apparent photosensory cells (lit. see Van Veen et al. 1984). Further, melatonin has been found to be localized in pinealocytes (lit., see Vollrath 1981, and authors of this volume). Since serotonin also occurs in CSF-contacting neurons it has to be questioned whether both cell types possess certain similarity in function. Concerning somatostatin and neurophysin we failed to demonstrate its presence immunocytochemically in the pineal complex of the eel, rainbow trout and European minnow. All these data indicate that immunocytochemical differences or similarities (serotonin) exist between CSF-contacting neurons and pineal photoreceptors. This hint proved to be of significance with regard to the antiopsin reaction.

Opsin in pinealocytes

Light microscopy. By means of sheep and rat antibodies raised against bovine opsin of rod outer segments of the retina a strong immunoreaction was observed in the outer segments of photoreceptors of the pineal and parapineal organs of the lamprey, Petromyzon fluviatilis, the cartilagenous fish the ray, Raja clavata, and a number of bony fishes, a.o. Anguilla anguilla, Salmo gairdneri, Phoxinus phoxinus, Gasterosteus aculea-





Figs 7 and 8. Opsin-immunoreactive outer segments of photoreceptors in the lumen (L) of the pineal organ of Rana esculenta and Phoxinus phoxinus. III 3rd ventricle, P pigment cells, SCO subcommissural organ. Semithin sections, antibovine (rhod)-opsin antibody, ABC method.

tus (Fig. 13). Similar results were obtained in photoreceptor outer segments of the frontal and pineal organs of amphibians (Fig. 7), of turtles and birds, further in retinas (Vigh and

Vigh-Teichmann 1981, Vigh et al. 1982, Vigh-Teichmann et al. 1980b, 1982, 1983a,b, Van Veen et al. 1984). These data reveal that pineal outer segments of these species contain (rhod)opsins, presumably associated with vitamin A-containing photopigments, as the corresponding retinas. Our results in the frog strengthen microspectrofluometric observations of a vitamin A_1 containing photopigment in the frog pineal organ (Hartwig and Baumann 1974), namely of a pigment with an absorption maximum at 502 nm corresponding to that of retinal rhodopsin. Further, our findings indicate that not only the pineal complex of lower vertebrates but also the pineal organ of reptiles and birds is involved in the direct perception of light. This assumption is in accord with neurophysiological results obtained in lower vertebrates and turtles (cf. Meissl and Dodt 1981).

With regard to mammals and some other species like lacertilians. the latter being known to contain photopigments in their pineal photoreceptors (cf. Meissl and Dodt 1981), our efforts failed to detect significant opsin immunoreactivity in the pinealocytes at the light microscopic level. This negative result does not exclude the possibility that the mammalian pineal organ elaborates some other kind of opsin-containing photopigment than demonstrable with our antibovine (rod)opsin antibody, and it may still have the capacity to detect light. This problem needs further studies with light and electron microscopic immunocytochemistry.

Interestingly, our light microscopic investigations on the pineal organ of cyclostomes, amphibians and certain teleosts not only revealed opsin-immunoreactive outer segments in the pineal organ proper, but also in its stalk (Fig. 8). In addition, in the frog, eel, rainbow trout and European minnow (often used species in behavioral and neurophysiological studies) opsin-immunoreactive outer segments of photoreceptors were observed inside the 3rd ventricle, in the orifice of the pineal recess and in the proximal thickened portion of the pineal stalk (Vigh-Teichmann et al. 1983b). This region (and the parapineal organ) may remain in connection with the brain in the case of conventional extirpation of the pineal organ, and percept light after distal pinealectomy. The proximal epithalamic pinealocytes may represent some of the extraretinal encephalic photoreceptors. In addition - quite recently - we could demonstrate opsin immunoreactivity in outer segments of extraretinal photoreceptors in the lumen of the optic nerve of the hagfish, Myxine glutinosa (Vigh-Teichmann et al. 1984a). This finding gives new impetus for the search for extraretinal photoreceptive elements.

Opsin immuno-electron-microscopy. At the electron-microscopic level, the antiopsin reaction was initially demonstrated on ultrathin sections of reembedded semithin-sectioned immunoreacted material (Vigh and Vigh-Teichmann 1981). It exhibited the usual signs of limited penetration of antibodies and did not allow to draw unequivocal conclusions on the presence of opsin-negative, i.e., non-immunoreactive outer segments. Recently, the protein A-colloidal gold labeling method

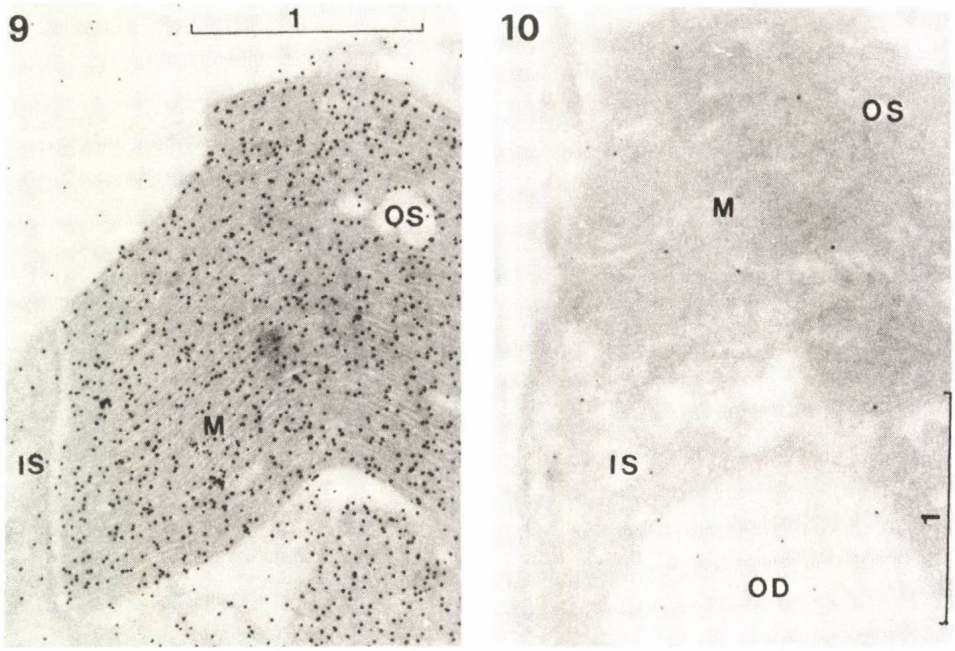


Fig. 9. Protein A-colloidal gold particles mark opsin-immunoreactive sites on "rod-like" outer segments (OS) of photoreceptors in the pineal organ of Rana esculenta. IS inner segment, M photoreceptor membranes. Ultrathin section, antibovine (rhod) opsin antiserum followed by antirat immunoglobulin.

Fig. 10. Opsin-negative "cone-like" outer segment (OS) of second photoreceptor class in the pineal organ of Rana esculenta. IS inner segment, OD oil droplet, M photoreceptor membranes. Ultrathin section, antibovine (rhod)opsin antiserum. A few protein A-gold particles correspond to background labeling.

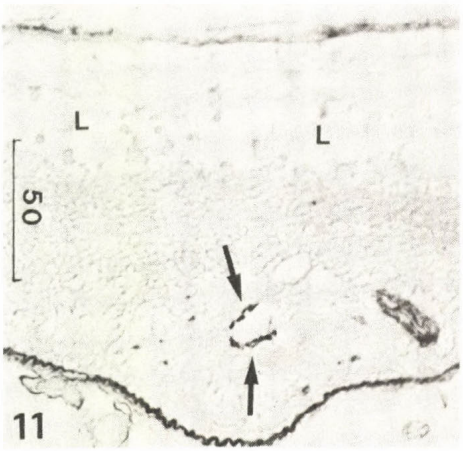
was used on ultrathin resin-embedded sections to visualize antigenic sites that bind antibovine (rhod)opsin antibodies in teleosts and amphibians (Vigh et al. 1983b, 1984, Vigh-Teichmann and Vigh 1983). We found numerous retinal rods and outer segments of pineal photoreceptors whose membrane discs were labeled with protein A-gold particles (Fig. 9). In the retina of the goldfish, the frog, the newt and the chicken certain cone-type outer segments did not show opsin immunoreactivity (Vigh et al. 1983b) with the above mentioned antiserum (see also frog retina: Papermaster et al. 1978; chicken retina: Szél et al. 1984). A similar result was obtained in the eye of the hagfish, Myxine glutinosa (Vigh-Teichmann et al. 1984a). The presence of opsin-negative outer segments in the retina shows that the various photopigments may be composed of different opsins and that not all opsins can be demonstrated with the antibovine (rhod)opsin antiserum used.

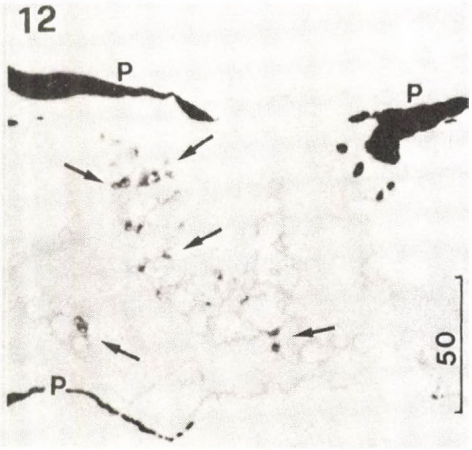
TWO CLASSES OF PHOTORECEPTOR CELLS

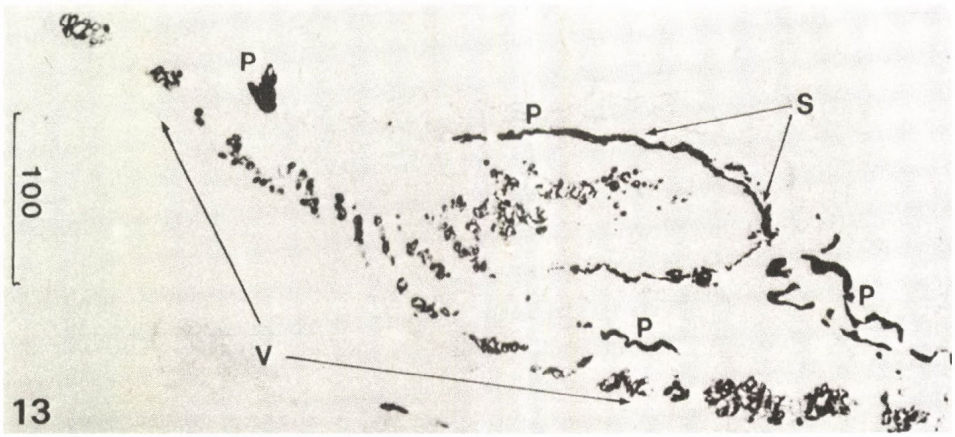
Quite recently, opsin-immunoreactive and non-immunoreactive outer segments (Figs 9,10) were detected in the pineal organ of the frog and the goldfish, but both classes of outer segments resembled cones morphologically (Vigh-Teichmann and Vigh 1983). In their immunocytochemical antigenicity the opsin-immunoreactive pineal outer segments resembled the retinal rods (rhodopsin), while the opsin-negative pineal outer segments exhibited antigenic properties like certain cones. Thus, the cone- or rod-like morphological appearance of photoreceptor outer segments does not answer per se the question what kind of opsin (photopigment) they may contain. In the frog, the class of opsin-immunoreactive pinealocytes appears to correspond to the pineal photoreceptors described microspectrofluorometrically to contain photopigment 502_1, a rhodopsin (Hartwig and Baumann 1974). Also electrophysiological studies indirectly indicated the presence of two types of photosensory cells (absorption maximum at 500 and 533 nm) in the pineal of teleosts (cf. Meissl and Dodt 1981).

In further ultrastructural and immuno-electron-microscopic investigations on the pineal organ of the frog, Rana esculenta, we found that the pineal photoreceptors endowed with opsin-positive or opsin-negative outer segments exhibited certain cytological differences (Vigh et al. 1984). The inner segments belonging to the opsin-negative outer segments contained an oil droplet (Figs 1,10). Such an oil droplet was lacking in the inner segments of the opsin-positive outer segments (Fig. 9). Earlier, Oksche and Vaupel-von Harnack (1965) had mentioned lipid droplets in certain pineal photoreceptors of the frog. Lipids were also described in the pinealocytes of a number of different vertebrates (lit., see Vigh et al. 1984). The presence of an oil droplet in the opsin-negative pinealocytes is a further parallel to the cones of the corresponding retina and strengthens the view that the frog pineal organ is composed of at least two classes of photoreceptors, of "rod-like" and "cone-like" ones.

By means of hybridoma supernatants, i.e., mono- or biclonal antibodies raised against isolated photoreceptor membranes of the retina of chickens (Szél et al. 1984) a small number of immunoreactive outer segments could be detected in the atrium of the pineal organ of the lamprey, Petromyzon fluviatilis (Fig. 11), further in the pineal organ of the European minnow, Phoxinus phoxinus (Fig. 12) and the frog, Rana esculenta. In the frog and the European minnow, these immunoreactive outer segments were scattered in the pineal organ (Fig. 12) in contrast to the close location of the abundant photoreceptors stained with the antibovine (rhod)opsin antiserum (Figs 7,13). For comparison, in the eel retina, numerous rods were positive while cones were negative (Fig. 14). In the pigeon retina, the immunoreactions with the monoclonal supernatants A_1 and D_6 demonstrated a varying number of cones while the rods remained unstained (Figs 15,16). The question has to be elucidated whether we are dealing with an opsin similar to iodopsin, the



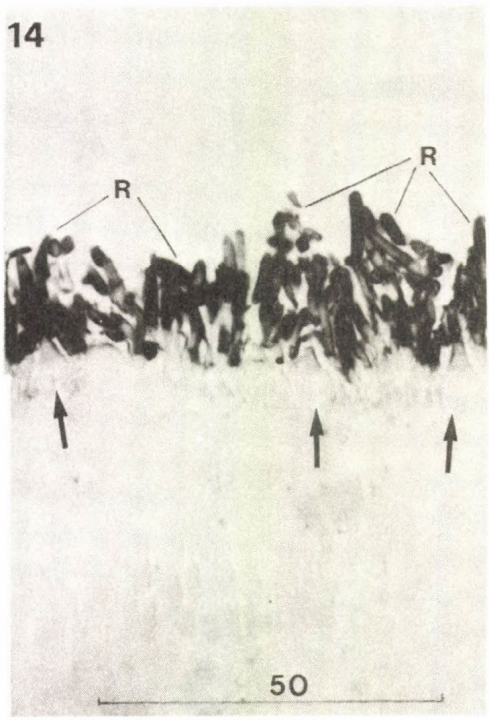


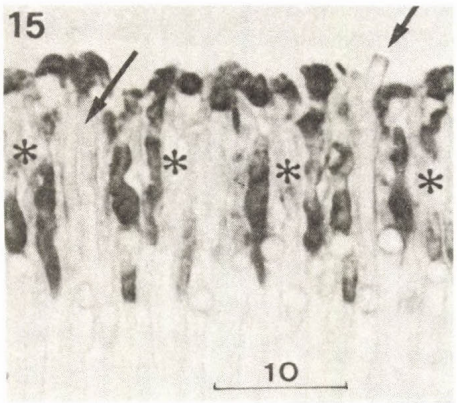


Figs 11 and 12. Second class of immunoreactive outer segments (arrows) of photoreceptors scattered in the pineal organ of the lamprey, Petromyzon fluviatilis, and the European minnow, Phoxinus phoxinus. L main lumen of lamprey pineal organ with non-immunoreactive outer segments, P pigment cells. Semithin sections, hybridoma supernatant against photoreceptor membranes of chicken retina, ABC method.

Fig. 13. Main class of opsin-immunoreactive outer segments in the endvesicle (V) and stalk (S) of the pineal organ of Phoxinus phoxinus. P pigment cells. Semithin section, antibovine (rhod)opsin antiserum, ABC method.

main photopigment of the chicken retina from which the antigen was prepared, or porphyropsin, the vitamin A_2 pigment with an absorption maximum at 533 nm demonstrated indirectly in the pineal organ of teleosts by extracellular recordings (cf. Meissl and Dodt 1981). At any case, by means of the different





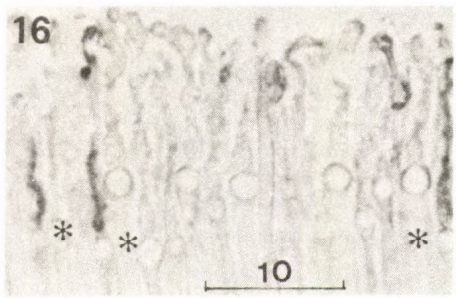


Fig. 14. Opsin-immunoreactive rods (R) and some opsin-negative cones (arrows) in the retina of the eel, Anguilla anguilla. Semithin section, antibovine (rhod)opsin antiserum, ABC method.

Fig. 15 and 16. Immunoreactive cone-type outer segments and non-immunoreactive rods (arrows) in the retina of the pigeon. Hybridoma supernatant A_1 (Fig. 15) marks cones with oil droplets in a more external retinal layer (asterisk) while supernatant D_6 (Fig. 16) selectively binds to cones containing oil droplets in a more internal position (small asterisk). Semithin sections, hybridoma supernatants against photoreceptor membranes of chicken retina, ABC method.

antiopsin antisera we have now immunocytochemical evidence of the presence of at least two classes of photoreceptors in the pineal organ of teleostean and amphibian species.

LANDOLT's CLUBS OF THE RETINA AS CSF-CONTACTING ELEMENTS

In the retina of many animal species and man, some perikarya of the layer of bipolar neurons dispose of dendrites that project toward the external limiting membrane. Here, the dendrites enlarge forming the so-called Landolt's clubs (1871). Landolt's clubs are endowed with small dendritic terminals (Fig. 1). They are supplied with solitary 9x2+0 cilia as characteristic of the CSF-contacting neurons of the hypothalamus.

At the electron microscopic level, we did not find any opsin immunoreactivity in these special structures of the retina. These data indicate that Landolt's clubs of the bipolar neurons have a greater similarity with the hypothalamic CSF-contacting neurons than with retinal photoreceptors. Finally, in this respect we have to note that under pathological conditions (ablation of the retina) the regressing photoreceptors themselves may become similar in structure to the periventricular CSF-contacting neurons of the hypothalamus (Vigh et al. 1983b).

FINAL CONCLUSIONS

Originally, we thought to get help in the research of the CSF-contacting neuronal system by comparing the CSF-contacting neurons with pineal and retinal photoreceptors. Contrary to our expectations, the comparative phylogenetic and immunocytochemical investigations rather elucidated structural peculiarities of the pineal organ and retina, i.e., they defined the (rhod)opsin content of photoreceptors of the pineal complex of a number of vertebrate species and the two classes of photosensory cells, rod- and cone-like ones, in the pineal organ. We hope that at a next conference on the pineal we can report that pineal research has given back the advantage it received from the intensive study of the CSF-contacting neuronal system for the benefit of the latter.

REFERENCES

Blähser S, Vigh-Teichmann I, Ueck M (1982) Cerebrospinal fluid-contacting neurons and other somatostatin-immunoreactive perikarya in brains of tadpoles of Xenopus laevis. Cell Tissue Res 224: 693-698

Grillner S, McClellan A, Sigvardt K (1982) Mechanosensitive neurons in the spinal cord of the lamprey. Brain Res 235: 169-173

Hartwig HG, Baumann C (1974) Evidence for photosensitive pigments in the pineal complex of the frog. Vision Res 14: 597-598

Hartwig HG, Oksche A (1982) Neurobiological aspects of extraretinal photoreceptive systems: structure and function. Experientia 38: 991-996

Landolt E (1871) Beiträge zur Anatomie der Retina vom Frosch, Salamander und Triton. Arch Mikr Anat 7: 81-100

Lengyel AJ, Grossman A, Nieuwenhuyzen-Kruseman AC, Ackland J, Rees J, Besser M (1984) Glucose modulation of somatostatin and LHRH release from rat hypothalamic fragments in vitro. Neuroendocrinology 39: 31-38

Leonhardt H (1980) Ependym und circumventriculäre Organe. In: Handbuch der mikroskopischen Anatomie des Menschen, Oksche A, Vollrath L (eds) Bd 4, Springer, Berlin Heidelberg New York, pp 177-666

Meissl H, Dodt E (1981) Comparative physiology of pineal photoreceptor organs. In: The pineal organ. Photobiology - Biochronometry - Endocrinology, Oksche A, Pévet P (eds), Developments in Endocrinology 14. 61-80, Elsevier, Amsterdam New York

Oksche A, Vaupel-von Harnack M (1965) Vergleichende elektronenmikroskopische Studien am Pinealorgan. Progr Brain Res 10: 237-258

Papermaster DS, Schneider BG, Zorn MA, Kraehenbuhl JP (1978) Immunocytochemical localization of opsin in outer segments and Golgi zones of frog photoreceptor cells. An electron microscope analysis of cross-linked albumin-embedded retinas. J Cell Biol 77: 196-210

Sano Y, Ueda S, Yamada H, Takeuchi Y, Goto M, Kawata M (1983) Immunohistochemical demonstration of serotonin-containing CSF-contacting neurons in the submammalian paraventricular organ. Histochemistry 77: 423-430

Szél A, Takács L, Monostori E, Vigh-Teichmann I, Röhlich P (1984) Heterogeneity of chicken photoreceptors as defined by hybridoma supernatants. An immunocytochemical study. Cell Tissue Res admitted

Underwood H, Groos G (1982) Vertebrate circadian rhythm: retinal and extraretinal photoreception. Experientia 38: 1013-1021

Van Veen Th, Ekström P, Nyberg L, Vigh-Teichmann I, Vigh B (1984) Serotonin and opsin immunoreactivities in the developing pineal organ of the three-spined stickleback. Cell Tissue Res in press

Vigh B, Vigh-Teichmann I (1971) Structure of the medullo-spinal liquor contacting neuronal system. Acta Morph Acad Sci Hung 19: 9-24

Vigh B, Vigh-Teichmann I (1973) Comparative ultrastructure of the cerebrospinal fluid-contacting neurons. Int Rev Cytol 35: 189-251

Vigh B, Vigh-Teichmann I (1981) Light- and electron-microscopic demonstration of immunoreactive opsin in the pinealocytes of various vertebrates. Cell Tissue Res 221: 451-463

Vigh B, Vigh-Teichmann I, Aros B (1975) Comparative ultrastructure of cerebrospinal fluid-contacting neurons and pinealocytes. Cell Tissue Res 158: 409-424

Vigh B, Vigh-Teichmann I, Aros B (1977) Special dendritic and axonal endings formed by the cerebrospinal fluid contacting neurons of the spinal cord. Cell Tissue Res 183: 541-552

Vigh B, Vigh-Teichmann I, Aros B, Sikora K, Jennes L, Simonsberger P, Adam H (1979) Comparative electron microscopy of medullo-spinal cerebrospinal fluid contacting neurons. Mikroskopie 35: 330-353

Vigh B, Vigh-Teichmann I, Olsson R (1980a) Development of the medullo-spinal cerebrospinal fluid-contacting neurons. In: Circulatory and developmental aspects of brain metabolism, Spatz M, Mrsulja BB, Rakic LjM, Lust WD (eds) Plenum Press, New York, pp 415-428

Vigh B, Vigh-Teichmann I, Sikora K, Jennes L (1980b) Scanning electron microscopy of the cerebrospinal fluid-contacting neurons of the central canal in various vertebrates. Verh Anat Ges 74: 707-714

Vigh B, Vigh-Teichmann I, Röhlich P, Aros B (1982) Immunoreactive opsin in the pineal organ of reptiles and birds. Z Mikr Anat Forsch 96: 113-129

Vigh B, Vigh-Teichmann I, Manzano e Silva MJ, Van den Pol AN (1983a) Cerebrospinal fluid-contacting neurons of the

central canal and terminal ventricle in various vertebrates. Cell Tissue Res 231: 615-621
Vigh B, Vigh-Teichmann I, Röhlich P, Oksche A (1983b) Cerebrospinal fluid-contacting neurons, sensory pinealocytes and Landolt's clubs of the retina as revealed by means of an electron-microscopic immunoreaction against opsin. Cell Tissue Res 233: 539-548
Vigh B, Vigh-Teichmann I, Oksche A (1984) Pinealocytes of "rod-" and "cone-type" characterized either by a selective antiopsin immunoreaction or by the presence of an oil droplet in their inner segment in the pineal organ of the frog (Rana esculenta). Cell Tissue Res in press
Vigh-Teichmann I, Vigh B (1974) The infundibular cerebrospinal -fluid contacting neurons. Adv Anat Embryol Cell Biol 50: 1-91
Vigh-Teichmann I, Vigh B (1979) Comparison of epithalamic, hypothalamic and spinal neurosecretory terminals. Acta Biol Acad Sci Hung 30: 1-39
Vigh-Teichmann I, Vigh B (1983) The system of cerebrospinal fluid contacting neurons. Arch histol jap 46: 427-468
Vigh-Teichmann I, Vigh B, Aros B (1970) Enzymhistochemische Studien am Nervensystem. IV. Acetylcholinesteraseaktivität im Liquorkontakt-Neuronensystem verschiedener Vertebraten. Histochemie 21: 322-337
Vigh-Teichmann I, Vigh B, Aros B (1976) Ciliated neurons and different types of synapses in anterior hypothalamic nuclei of reptiles. Cell Tissue Res 174: 139-160
Vigh-Teichmann I, Vigh B, Aros B, Jennes L, Sikora K. Kovács J (1979) Scanning and transmission electron microscopy of intraventricular dendrite terminals of hypothalamic cerebrospinal fluid contacting neurons in Triturus vulgaris. Z Mikr Anat Forsch 93: 609-642
Vigh-Teichmann I, Vigh B, Aros B (1980a) Ciliated perikarya, "peptidergic" synapses and supraependymal structures in the guinea pig hypothalamus. Acta Biol Acad Sci hung 31: 373-394
Vigh-Teichmann I, Röhlich P, Vigh B, Aros B (1980b) Comparison of the pineal complex, retina and cerebrospinal fluid contacting neurons by immunocytochemical antirhodopsin reaction. Z Mikr Anat Forsch 94: 623-640
Vigh-Teichmann I, Vigh B, Aros B, Kausz M, Simonsberger P, Van den Pol AN (1981) CSF contacting neuronal structures of the third ventricle of opossum, hedgehog and cat. Mikroskopie 38: 337-355
Vigh-Teichmann I, Korf HW, Oksche A, Vigh B (1982) Opsin-immunoreactive outer segments and acetylcholinesterase positive neurons in the pineal complex of Phoxinus phoxinus (Teleostei, Cyprinidae). Cell Tissue Res 227: 351-370
Vigh-Teichmann I, Vigh B, Manzano e Silva MJ, Aros B (1983a) The pineal organ of Raja clavata: Opsin immunoreactivity and ultrastructure. Cell Tissue Res 228: 139-148
Vigh-Teichmann I, Korf HW, Nürnberger F, Oksche A, Vigh B, Olsson R (1983b) Opsin-immunoreactive outer segments in the pineal and parapineal organs of the lamprey (Petromyzon fluviatilis), the eel (Anguilla anguilla) and the rainbow trout (Salmo gairdneri). Cell Tissue Res 230: 289-307

Vigh-Teichmann I, Vigh B, Korf HW, Oksche A (1983c) CSF-contacting and other somatostatin-immunoreactive neurons in the brains of Anguilla anguilla, Phoxinus phoxinus, and Salmo gairdneri. Cell Tissue Res 233: 319-334

Vigh-Teichmann I, Vigh B, Olsson R, Van Veen Th (1984a) Opsin-immunoreactive outer segments of photoreceptors in the eye and the lumen of the optic nerve of the hagfish, Myxine glutinosa. Cell Tissue Res in press

Vigh-Teichmann I, Van Veen Th, Vigh B (1984b) 5-HT immunoreactivity in the brain and spinal cord of the hagfish (Myxine glutinosa) with special reference to CSF-contacting neurons. In preparation

Vollrath L (1981) The pineal organ. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd 6/7, Oksche A, Vollrath L (eds), Springer, Berlin Heidelberg New York

PINEAL HORMONES AND REGULATORY MECHANISMS OF PINEAL SECRETION

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

CELLULAR BIOLOGY OF THE PINEAL ORGAN WITH SPECIAL REFERENCE TO SOME KNOWN AND OTHER HYPOTHETICAL MESSENGER SUBSTANCES; THE CRL CONCEPT AND OUTLOOK

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To the memory of Dr M.T. JUILLARD, a very regretted dear friend.

CONTENTS

"Long ago it became evident that the key to every biological problem must finally be thought in the cells, for every living organism is, or at some time has been, a cell".

E.B. WILSON, 1925.

INTRODUCTION

A general feature of the pineal organ of vertebrates is to translate (i) photoperiodic stimuli, and (ii) various forms of exogenous and endogenous stimuli into informational molecules, their levels being characterized by cyclic changes. Some of these messengers are believed to be involved in the temporal organization of physiological processes.

Among the major unsolved questions in pineal research, are (i) the cellular mechanisms of this translation, and (ii) the mode of local interaction and communication of pineal cells with extrapineal targets.

As reviewed recently (Collin and Oksche, 1981, and previous reports),

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the principal pineal cells, named "cells of the receptor line" (CRL), correspond to cone-like photoreceptor cells and their phylogenetic derivatives.

CRL, responding to environmental stimuli, may operate in different ways, depending on their state of differentiation in vertebrate series, i.e., by an

- output of neurotransmitter(s) : the signal is passed to the brain via afferent (pinealofugal) neurons which establish a pineal tract ;
- and/or output of bioactive compounds that might act locally and/or influence extrapineal targets via the bloodstream and the cerebrospinal fluid (CSF).

Efferent (= pinealopetal) peripheral and central axons convey exogenous and endogenous stimuli. They employ different messenger compounds to influence the pineal organs, probably via the CRL.

Since indoles and hypothetical peptides are (or may represent) biologically potent molecules of the pineal organ, the sites of indole metabolism and protein secretion will be examined (Sections I & II) to come to some conclusions concerning the physiology of CRL (Section III) and of certain efferent axons (Section II). Thereafter, I shall present my interpretation of the CRL concept (Section IV).

For a better understanding of the following, the reader is referred to a monograph edited by Reiter (1981), dealing with cytological and biochemical aspects (for indole metabolism, see also Balemans, 1981 ; for innervation, Ueck, 1979, Korf and Møller, this volume).

I. SITES OF INDOLE METABOLISM IN THE PINEAL ORGAN OF VERTEBRATES

A. MOST CRL ARE SEROTONERGIC ELEMENTS

5-hydroxytryptamine (or serotonin = HT), the precursor of a large number of indoles found in the pineal, and/or 5-hydroxytryptophan (HW) have been detected by means of cytochemical techniques. Because HT is an indoleamine, more correctly an indolylalkylamine, the cells involved in its synthesis, storage and release may be named indoleaminergic (or serotonergic) elements.

LIST OF ABBREVIATIONS USED IN THE TEXT :

aHT : N-acetyl-5-hydroxytryptamine (= N-acetyl-serotonin)
aML : O-acetyl-5-methoxytryptophol
aMT : N-acetyl-5-methoxytryptamine (= melatonin)
AVT : Arginine vasotocin
CIL : Cells of the interstitial (= ependymal) line (= interstitial cells)
CP : Non-indolergic cone-like photoreceptor cell
CRL : Cells of the receptor line
CSF : Cerebrospinal fluid
DCV : Dense-cored vesicles (= secretory granules)
EM : Electron microscopy
HA : 5-hydroxyindole-3-acetic acid
HT : 5-hydroxytryptamine (= serotonin)
HW : 5-hydroxytryptophan
HL : 5-hydroxytryptophol
ICP : Indolergic cone-like photoreceptor cell
LH-RH : Luteinizing hormone releasing factor
MA : 5-methoxyindole-3-acetic acid
ML : 5-methoxytryptophol
MP : Modified photoreceptor cell
α-MSH : α-melanocyte stimulating hormone
MT : 5-methoxytryptamine
MW : 5-methoxytryptophan
Pi : Pinealocyte
RIA : Radioimmunoassay
VIP : Vasoactive intestinal peptide
HMP : Highly modified photoreceptor cell
W : Tryptophan

The pool of HW/HT has been demonstrated in numerous but not in all investigated species (Collin, 1979 ; Møller and Van Veen, 1981 ; Meiniel, 1981 ; Juillard and Collin, 1980 ; Van Veen et al., 1980 ; Hartwig and Reinhold, 1981 ; Voisin et al., 1982). Species-dependent differences were detected, resulting in a distribution of this pool either in CRL or in both CRL and cells of the ependymal (= supportive or interstitial) line (CIL) (Collin, 1979 ; Collin and Oksche, 1981). In addition, at least in some species, orthosympathetic (noradrenergic) fibers take up HT which is released from CRL and/or CIL. All these data were obtained by means of the Falck-Hillarp technique and microspectrofluorometry.

By use of the argentaffin and chromaffin reactions in control and drug-treated animals, HT stores were identified electron-microscopically in the pineals of the wall lizard, the parakeet and the mouse. It was concluded that dense-cored vesicles (= DCV ; = secretory granules) of rudimentary (= modified) photoreceptor cells of lizard and parakeet as well as those of mouse pinealocytes store HT which coexists with a protein secretion (ref. in Collin, 1979).

In addition, it appeared that HT constitutes mainly a granular pool in sauropsids and, in contrast, an extragranular cytoplasmic pool in the mouse. By use of the Falck-Hillarp technique, Meiniel (1981) concluded that in the lamprey HT occurs mainly in the residual bodies of a certain category of cone-like photoreceptors.

By use of the routine technique of radioautography, the in-vitro or in-vivo uptake of tritated tryptophan (^{3}H-W), ^{3}H-HW and ^{3}H-HT was investigated in all classes of vertebrates (ref. in Collin, 1979 ; Collin and Oksche, 1981 ; Balemans et al., 1983). Taking into consideration that indoles are diffusible substances, later experiments (see below) suggest that, in principle, the previous data (i) are correct for birds and reptiles, (ii) must be re-evaluated or even revised in Anamnia, and (iii) cannot be accepted, except for orthosympathetic nerve terminals, in mammals. Without going here into details it appears that results obtained with radioautography are in line with most of the evidence obtained in the same species with the use of the Falck and Hillarp technique.

Together with these findings, pharmacological and biochemical data enabled to come to the following conclusions :

(i) : CRL (pinealocytes, modified = rudimentary photoreceptor cells and - at least according to Meiniel (1981) - one of the categories of cone-like photoreceptors) (Fig. 1) synthesize, store and release HT. Thus CRL are serotonergic cells. The metabolism of HT in CRL was always strongly suggested.

(ii) : CIL may store and probably deaminate or release HT.

(iii) : occasionally in amniotes, orthosympathetic nerve fibers take up HT released from CRL and CIL ; furthermore central serotonergic nerve fibers were also found (Matsuura and Sano, 1983).

In the pineal organ it is well known that HT is the precursor of active 5-methoxyindoles (melatonin = aMT, a substituted amide ; 5-methoxytryptophol = ML, an alcohol ; 5-methoxytryptamine = MT, an indoleamine) (Section III, A). Thus, the following investigations were focussed on checking the hypothesis according to which the serotonergic CRL are "indolergic". "Indolergic" means that all steps of indole metabolism may be found in the same cell and that indoles may be stored in and released from this cell type.

B. INDOLERGIC CRL, AS DEMONSTRATED IN SOME VERTEBRATES

With the alternating use of several biochemical and cytochemical techniques, very recently new results were obtained for CRL of some submammalian vertebrates. Data in mammals are only acceptable for efferent orthosympathetic nerve fibers.

1. Birds and lizards

Since indoles are known to be diffusible substances, their retention in pineal tissue was examined in the parakeet during the preparation of specimens for EM. Following in-vivo uptake of (^{3}H)-HW and in-vitro uptake of (^{3}H)-HT, the radioactivity of fluids used in the processing of pineals was determined by liquid scintillation counting. No exogenous (^{3}H)-indoles could be revealed in EM-solutions after (^{3}H)-HW in-vivo uptake ; 8.8 to 13.4 % of (^{3}H)-indoles were washed out by glutaraldehyde (fixative) after (^{3}H)-HT in-vitro uptake (Balemans et al., 1983). On the contrary, most indoles were lost after (^{3}H)-aMT uptake in-vitro but, under these conditions, the specificity of the uptake is also to be questioned.

In the parakeet, experiments were carried out in the autumn during the first hours of the scotophase, because under these conditions the methylation of hydroxyindoles for the production of melatonin/5-methoxytryptophol (not separated in the chromatographic system used) reached its maximum (Balemans et al., 1981). In the pigeon, the experiments were performed during the scotophase, mainly at the time when a peak of aMT was detected by radioimmunoassay (= RIA ; Voisin et al., 1982).

a. Pineal organ of the parakeet

In a first in-vivo autumnal experiment using the parakeet (Collin et al., 1982), the animals were injected i.p. with (^{3}H)-HW (i) at the onset of darkness, then maintained in darkness and killed 15, 45, 60 min later, or (ii) during the first three hours of darkness and then killed after 60min. From the detailed qualitative and quantitative chromatographic study of indoles produced from (^{3}H)-HW, it appears that the precursor was metabolized to 5-hydroxytryptamine/5-methoxyindole-3-acetic acid (HT/MA), N-acetyl-5-hydroxytryptamine (aHT), 5-hydroxytryptophol (HL), MT, 5-methoxytryptophan/5-hydroxyindole-3-acetic acid (MW/HA), melatonin/5-methoxytryptophol (aMT/ML). aMT/ML, the main metabolites of HT, increased with time after onset of darkness. Whatever the experimental series and pineal portions, tissue sections treated for radioautography displayed a high labeling in most of the modified photoreceptor cells, characterized by the presence of DCV. Other cell types were never significantly labeled.

In a second in-vivo experiment carried out in summer, parakeets were injected i.p. with (^{3}H)-HW at different times of the 24h light/dark (16L/8D) cycle (in preparation). Irrespective of the time of treatment, during the photophase or the scotophase, radioautography combined with thin layer chromatography showed that modified photoreceptor cells are capable of the biosynthesis of 5-methoxyindoles (MW, MT, MA, ML, aMT and O-acetyl-5-methoxytryptophol : aML). The amounts of these indoles displayed a circadian rhythm except that of aML and MA, which were always low ; aMT reached its maximum level during the dark phase.

In parallel to the autumnal in-vivo experiment (see above), five

groups of isolated pineal organs were incubated in-vitro, just at the beginning or within the first hours of the scotophase, with a 20min pulse of (^{3}H)-HT, then postincubated for 15, 30, 45 and 55min with a radioinert medium (Juillard et al., 1983). From the qualitative and quantitative chromatographic study of indole compounds produced from (^{3}H)-HT, the synthesis of HT/MA, aHT, HL, MT, aMT/ML and HA was clearly evident. (^{3}H)-metabolites were also released into the post-incubation medium. Although some quantitative biochemical differences were found between the in-vitro and in-vivo experiments, only modified photoreceptor cells displayed selective radioautographic reactions.

b. Pineal organ of the pigeon

In several in-vitro experiments conducted during the spring season (Voisin et al., 1983 and ref.), the cell type responsible for the nocturnal indole metabolism was identified. After a short-term incubation or organ culture in the presence of (^{3}H)-HW or (^{3}H)-HT, the relative amounts of deaminated and acetylated indoles were measured by the use of chromatography with or without application of inhibitors of monoamine oxidase and cyclic nucleotide phosphodiesterase. Irrespective of the experimental conditions, radioautography combined with radiobiochemistry showed that only modified photoreceptor cells display a significant labeling.

c. CRL (here modified photoreceptor cells) are the indolergic cells in the pineal organs of the parakeet, the pigeon and the common lizard

In the in-vivo experiments the long radioactive signal (up to 60min, after i.p. injection of (^{3}H)-HW) was of particular interest (see discussion in Collin et al., 1982). Taking into account the fact that the metabolism of (^{3}H)-HW, as shown biochemically, paralleled a permanent uptake of this precursor and that the selective radioautographic reactions were always and exclusively found in CRL, it was assumed that these cells take up, retain and metabolize (^{3}H)-HW from the very beginning onset. All steps of indole metabolism appear to be present in CRL. The storage sites of exogenous indoles correspond to those of endogenous HT, as can be demonstrated by means of the Falck-Hillarp technique (Juillard et al., 1977 ; Voisin et al., 1982). Indoles are produced and stored in the perikaryon, mainly in the branched basal pedicles of the CRL of the parakeet. As suggested from other experiments (Ralph, 1976), CRL probably release indole metabolites directly into the bloodstream. In the pigeon, indoles are found in the different compartments of the CRL, except in the outer segment. In addition, it has been noted that the retention of these products during the experimental procedure was excellent in-vivo and good in-vitro ; indoles were found in CRL over the 24h-cycle.

In sum these results allow to conclude that modified photoreceptor cells of the parakeet and the pigeon are indolergic. Taking into account that in preliminary experiments with the common lizard (Collin, 1979 and unpublished), the radioautographic reactions after (^{3}H)-HW in-vivo uptake were high and selective at different times of the 24h-cycle, a similar conclusion is derived for the rudimentary (= modified) photoreceptor cells of this reptile (see also Collin, 1979).

2. Teleost fish (pike)

Several differing methodologies have been used by Falcon et al. (see

Falcon, 1984) to demonstrate that CRL (i.e., cone-like and modified photoreceptor cells) are indolergic.

The storage sites of endogenous indoles were investigated with or without pharmacological treatments. HW/HT (and possibly MW and MT) were visualized by means of the Falck-Hillarp technique. Fluorophores were mainly distributed in CRL, particularly in their inner segments, cell bodies and in rosettes corresponding to aggregates of photoreceptor cell pedicles. Using antisera against HT, aHT and aMT, the immunoreactive light- and electron-microscopically detected products were confined to the two types of CRL. However, low levels of an aMT-like immunoreactivy were also present in CIL at least at the beginning of the photophase. In addition, cytochemistry and RIA allowed to show circadian variations of the concentrations of HT, aHT, aMT and MT (Falcon, 1984, for more details). Since HT, aHT and aMT were found only or mainly in CRL, it was strongly suggested that CRL not only store but also synthesize these indoles. The following complementary experiments support this view :

From in-vitro experiments using thin layer chromatography, the metabolism of exogenous indoles, (^{3}H)-HT and (^{3}H)-aMT, was investigated (Falcon, 1984). The uptake and metabolism of (^{3}H)-HT resulted in the formation of HA, HW, MW, HL and ML ; the concentrations of MT, aHT, aMT and MA were low or very low. The radioautographic labeling, found concurrently, was mainly present in both types of CRL. (^{3}H)-aMT was converted mainly in (^{3}H)-ML, possibly after deacetylation of (^{3}H)-aMT, followed by the oxidative deamination of the neosynthesized (^{3}H)-MT. Although biochemical complementary data are necessary in fish, Falcon (1984) suggests that the pathways of indole metabolism may be more complex than those previously described in the pineal of birds and mammals.

In-vivo uptake of (^{3}H)-W resulted in a labeling mainly or exclusively present in CRL (Falcon et al., 1980). After in-vivo and in-vitro uptake of (^{3}H)-HW, silver grains were distributed in all CRL and CIL or mainly in CRL, depending on the state of inhibition of the oxidative deamination pathway (Falcon et al., 1980).

Taking into account (i) the sites of some enzymatic activities, (ii) a partial extraction of indoles during the processing of pineals for cytology, and (iii) a partial diffusion in CIL, Falcon (1984) discusses the significance of the reactions after uptake of the (^{3}H)-indoles.

From this body of complementary data for the pineal organ of the pike, Falcon (1984) comes, for the first time in Anamnia, to the conclusion that cone-like and modified photoreceptor cells are indolergic. These CRL synthesize and store indoles which are probably released into the bloodstream and CSF. The reader is also refered to a pertinent review on the pineal organ of fishes by McNulty (1984).

3. Mammals (rat, rabbit and hamster)

The validity of the labeled sites after in-vitro uptake of (^{3}H)-HT was examined in the pineal of the rat by the use of high resolution radioautography, chromatography and liquid-scintillation counting (Juillard et al., 1984).

During the scotophase, MT, aHT, aMT/ML, HA, MA, HL, aML were formed from (^{3}H)-HT. The corresponding selective radioautographic reactions were observed in the sympathetic nerve terminals, but were missing after bilateral surgical removal of superior cervical ganglia. In contrast, a

scarce and diffuse labeling was distributed in pinealocytes and interstitial cells of both untreated and ganglionectomized rats. Similar radioautographic reactions were obtained in the hamster and the rabbit.

The fixative and other fluids used in the radioautographic routine technique were inadequate for the visualization of the sites of indole metabolism in the mammalian pineal cells. The loss of indoles (57 %) was considerable, in comparison to the above-studied vertebrates, when similar techniques were used. Indole binding molecules (e.g., indole binding proteins) were suggested to be responsible for the species-dependent differences among vertebrates.

C. CONCLUSIONS CONCERNING CRL, CIL, AND SYMPATHETIC AND CENTRAL AXONS

The above-mentioned recent results, obtained at the cellular level, allowed to increase substantially our knowledge on indolergic cells. Unfortunately, so far only few nonmammalian species have been examined. All steps of indole metabolism depend on an enzymatic mechanism in CRL (i.e. : cone-like photoreceptors in the pike, and modified photoreceptor cells in the pike, lizard, parakeet and pigeon) (Collin, 1979). The compartments of CRL, involved in indole metabolism, have been outlined (Collin, 1979 ; Collin et al., 1982 ; Juillard et al., 1983 ; Voisin et al., 1983 ; Falcon, 1984). Most enzymes are active in the cytosol, except monoamine oxidase which is associated with the outer membrane of mitochondria, the membranes of the endoplasmic reticulum, and also those of the nuclear envelope and (mainly in the pike) the plasma membrane (Juillard and Collin, 1979 ; Falcon, 1984). Tryptophan-5-mono-oxygenase is apparently not present in mitochondria (ref. in Collin, 1979 ; Hamon, personal communication). The use of antibodies against enzymes of indole metabolism may lead to a greater precision concerning the distribution of enzymes during a 24h-cycle. However, ultracytochemical techniques for detection of the sites of these activities will be most important.

The function of CIL is not fully understood although they are, at least in some species (e.g. in the pike), involved in the uptake of indole precursor(s), preceeding that in the CRL. A diffusion of metabolites (e.g. HT) from CRL to CIL was also hypothesized (Collin, 1979). In CIL the oxidative deamination serving the production of HA is very apparent (Collin, 1979). A study on the distribution of enzymatic activities will be very useful for clarification of the role of CIL in indole metabolism.

The functional significance of the uptake of HT by orthosympathetic nerve terminals in some avian and mammalian species remains apparently uninterpreted. In this context the role of central serotonergic nerve fibers is unknown.

II. CYTOLOGICAL ASPECTS OF PROTEIN SECRETION IN THE PINEAL ORGAN OF VERTEBRATES

This survey will concentrate exclusively on hypothetical exportable peptides. Since this topic has been reviewed recently, only a brief comment will be made (Collin, 1977, 1979, 1981 ; Collin and Oksche, 1981 ; Pévet, 1983 and ref.).

As a rule, peptides may be found in all pineal cells. To date, the studies were mainly focussed on CRL, CIL and efferent axons.

A. CRL and CIL

With the use of bioassay, RIA and immunocytochemistry, substance(s) immunologically similar to certain peptides have been reported to occur mainly in the mammalian pineal (ref. in Pévet, 1983 ; Rix et al., 1981). With antisera against LH-RH, α-MSH, somatostatin, AVT, angiotensins I and II, angiotensinogen, very similar, if not identical, staining patterns were observed in the rat (Rix et al., 1981). Several cytologists thought that this staining is confined to unidientified parenchymal cells and their processes, diffusely distributed throughout the gland. On the contrary, Rix et al. (1981) suggest that these immunoreactions were restricted to the extracellular perivascular compartment, an interpretation partly accepted by Pévet (1983) (see also Piekut and Knigge, 1982). According to some authors, LH-RH is present only in efferent fibers (see below). All these authors argue that the antibodies cross react with compounds which are not identical to the genuine peptides. In this context, it is believed by several groups (ref. in Pévet, 1983) that the identification of AVT, previously considered as a mammalian pineal hormone, is an error. However, Prechel et al. (1983) conclude that no data are available to prove or disprove the tentative identification of AVT.

Thus, immunocytochemistry did not give clear-cut evidence for the occurence of one of the known neuropeptides in CRL and/or CIL.

Nevertheless, from ultrastructural observations and ultracytochemistry it is evident (Collin, 1977 ; 1979, 1981 ; Collin and Oksche, 1981 ; Pévet, 1983) that modified photoreceptor cells and pinealocytes (and possibly cone-like photoreceptors) are capable of protein secretion. The endomembranous system (i.e. the complex rough endoplasmic reticulum → Golgi apparatus → DCV) is, in analogy to other peptidergic neurons or endocrine cells, involved in protein secretion. Collin (1981) postulated that (a) specific peptide(s) termed "pinealin(s)" stored in the secretory granules arise from the processing of a precursor, or possibly several precursors in the endomembranous system. The neurohormonal activity of this (these) peptides is still purely hypothetical. The cytological studies on the influence of different agents, which strengthen the cytological data on protein secretion, do not belong to the scope of this survey (see Pévet, 1983 and following papers). Finally, although there exists also some indication of a protein secretion in CIL, the data are still not coherent enough to be reviewed here.

B. EFFERENT PEPTIDERGIC-LIKE AXONS OR EFFERENT MULTIMESSENGER AXONS ?

Functional interelationships between central nervous system and the pineal organ have been suggested by several investigators of efferent neurons (Ueck, 1979 ; Korf and Møller, this volume).

Several authors have reported that efferent axonal terminals, filled with selectively stainable ("Gomori-positive") neurosecretory material, or "neurosecretory fibers" displaying elementary granules in EM, occur in the pineal of a number of vertebrate species. Recently, antisera generated against some proteins and peptides revealed immunoreactive material in certain nerve fibers of higher vertebrates.

In the rat, oxytocin- and vasopressin- immunoreactive fibers originating - at least partly - from the magnocellular nuclei, were traced up to the level of the stalk and anterior part of the pineal (Buijs and Pévet, 1980 : ref. in Pévet, 1983). In the hedgehog (Nürnberger and Korf, 1981), similar fibers emerge from the dorsal portion of the paraventricular nucleus and then enter the pineal organ where they mainly occur in the

central portion and secondarily at the periphery. The majority of these fibers is intimately related to the capillaries, whereas only few elements are scattered throughout the pineal parenchyma. Similar observations were also reported in the dog (Matsuura et al., 1983). In rat, hedgehog and dog, these fibers enter the pineal gland via the habenular commissure and in juxtaposition with the subcommissural organ. Substances immunologically resembling neurophysin (pigeon), LH-RH (squirel monkey ; dog), somatostatin (rat), substance P (cat), VIP (cat, pig, rabbit) were also found in certain fibers (ref. in Pévet, 1983 ; Matsuura et al., 1983).

These first data on the storage sites of some peptides suggest that nerve fibers of central origin could play a role, e.g., in the control of the messages delivered by pineal cells, or in local blood flow and/or in effects on distant targets.

Additional comparative ultrastructural and ultracytochemical studies would be welcomed. What are the different types of pineal efferent fibers ? Does each type employ several messenger compounds (one or more peptides and small molecules, i.e. amino acids, amines, acetylcholine) ? There is mounting evidence that endocrine cells as well as neurons harbour in their vesicles or granules a set of substances including several messengers and occasionally more than one messenger peptide. Peptides arise from the processing of one or several precursors produced in the same cell type.

In summary, studies applied to efferent neurons have barely begun. The messengers of efferent fibers might communicate locally or at distance. A distinction between neurotransmitters, neuromediators and possibly neurohormones is also needed (Nürnberger and Korf, 1981).

III. CRL : INFORMATIONAL MOLECULES AND PHYSIOLOGY

A. GENERAL CONSIDERATIONS (PINEAL FUNCTION)

Pineal organs are primarily concerned with adaptation to environment ; in this connection direct responsiveness to light remains their sine qua non (Ralph, 1983). They are involved in temporal organization of physiological processes and represent a component in one of the circuits by which photoperiod synchronizes circadian rhythms (Ralph, 1983). Concerning the many functions ascribed to pineal organs, there are differences among groups of vertebrates. The photoperiodic information is translated into signals that are most "advantageous" to the species, e.g., the pineal affects reproduction apparently only in a number of species.

Although the sites of action of neurosensory and hormonal messages of pineal organs may be different, many of these effects are mediated by specialized regions of the brain.

The afferent neurons postsynaptically linked by means of (a) neurotransmitter(s) to cone-like photoreceptors, project in teleosts and anurans to the reticular formation of the brainstem and may serve phototactic reactions, the control of thermoregulatory behaviour and some unknown activating mechanisms. The hypothalamic projection may be involved in circadian and neuroendocrine functions (Oksche, 1984).

aMT, often considered as a potential pineal hormone, acts at different sites, but many of its effects are mediated by the hypothalamus. This indole is involved in a number of well elucidated functions (reproduction, thermoregulation, circadian rhythmicity) and in color changes (Ralph et al., 1979 ; Ralph, 1983 ; Reiter, 1983 ; McNulty, 1984). According to Ralph (1983) aMT is a key molecule affecting adaptation to annual events,

in invoking appropriate "programs" which permit an animal to anticipate seasonal changes. Besides aMT and ML (apparently released from pineals), MT deserves mention as possible hormonal agent (Reiter, 1983). According to Reiter (1983), the data concerning pineal peptides, mainly studied in mammals, remain open to discussion.

B. CRL AND TRANSLATION OF PHOTOPERIODIC STIMULI INTO INFORMATIONAL MOLECULES

1. Cone-like photoreceptor cells (Fig. 1)

The presence of these cells, established in petromyzontids, fishes, amphibians, chelonians and lacertilians, still remains hypothetical in birds.

a. Non-indolergic cone-like photoreceptor cells (= CP)

According to Meiniel (1981), it was not possible to visualize the presence of HT in CP of the brook lamprey. In the common lizard (ref. in Collin, 1979 and Meiniel, 1981) and the lamprey (Meiniel, 1981), an uptake of (^{3}H)-5-HTP and/or retention of its metabolites in CP was not significant.

The interpretation of these results requires complementary investigations. There are two alternatives : either these cells are really not serotonergic, or they are devoid of this material due (i) to a rapid metabolic breakdown or release of HW/HT, and/or (ii) to the limitations of the fluorescence and radioautographic techniques (Collin, 1979 ; Juillard et al., 1984 ; Falcon, 1984).

Complementary experiments appear to be necessary before concluding definitively that in some pineal organs transducers exist which can produce only neural messages, by means of an unidentified neurotransmitter delivered at the ribbon synapses, in response to photoperiodic information (Collin, 1977). This unknown neurotransmitter is supposed to be stored in electron-lucent synaptic vesicles (Collin, 1969). In addition, DCV which store a protein secretion in CRL of amniotes are also present in the CP of the lamprey and the lizard (Collin, 1969). Therefore, the present data are still incomplete to dismiss definitively (i) the presence of informational molecules different from a neurotransmitter, and (ii) a possible secretory (endocrine ?) role of CP (Collin, 1971).

b. Indolergic cone-like photoreceptor cells (= ICP)

Typical cone-like photoreceptor cells, as shown in the brook lamprey, some fishes and apparently also in the frog store HT (Section I). The complementary experiments of Falcon (1984) enable to conclude in the pike, that they are indolergic (Section I).

Although aMT has been considered as a potential hormone in several groups of vertebrates, it was mentioned that this informational molecule (and possibly other 5-methoxyindoles) may be employed also as parahormone or neurotransmitter, depending on the tissue or functional demand (Ralph, 1983 ; Oksche, 1984). Gern (ref. in Ralph, 1983) postulated also that aMT, a characteristic product of ciliated photoreceptors, has an action within these cells.

Accepting that aMT (and possibly other 5-methoxyindoles) correspond to

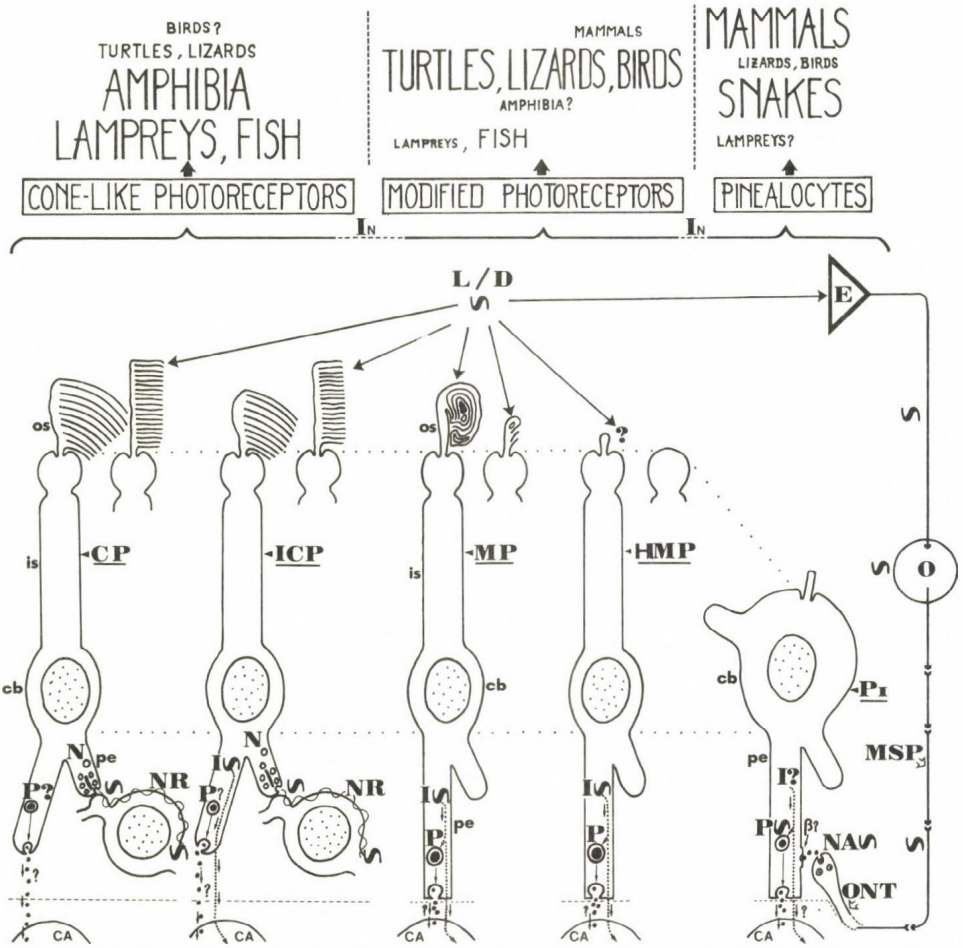

FIG. 1. Principal cell types of the receptor line (CRL) : CP, ICP, MP, HMP and Pi (see list of abbreviations), In (intermediate cell types). Note the distribution of the different types of CRL in the individual classes or orders of vertebrates.

INPUTS : Direct transduction of photoperiodic information (Light/Dark : L/D) may occur in CP, ICP and MP. Indirect transduction takes place only in Pi (B : B-receptor ; E : eye ; NA : noradrenaline ; MSP : multisynaptic pathway ; O : oscillator ; ONT : orthosympathetic nerve terminal).

OUTPUTS : The physiological activity of the cells varies in a periodic manner (period of, or close to, 24h : ∽). Three messenger substances are indicated.

. I : only ICP, MP and HMP of a number of species have been shown to be indolergic (see text).

. N : corresponds in CP or ICP to an unknown neurotransmitter. The signals are passed to the brain via afferent neurons (NR).

. P : proteic secretory material (peptides : pinealins) located in secretory granules ; its release from CRL remains to be definitively established in most vertebrates.

(ca : capillary ; cb : cellular body ; is : inner segment ; os : outer segment ; pe : synaptic or asynaptic process).

(a) neurohormone(s) (see also de Vlaming and Olcese, 1981 ; McNulty, 1984), but not excluding other functions, Falcon (1984) emphasizes that ICP in the pike are photoneuroendocrine cells.

The day/night changes of (i) indole contents (Falcon, 1984 and ref.), (ii) enzymatic activities implicated in indole metabolism (ref. in McNulty, 1984 ; Falcon, 1984), and (iii) electrical responses (Falcon and Meissl, 1981) permit to conclude that ICP of the pike translate photoperiodic stimuli into two categories of signals :

(i) a signal is passed to the brain by means of an unknown neurotransmitter released at the ribbon synapses between ICP and afferent neurons ;
(ii) indolic signals (i.e. 5-methoxyindoles) which, in addition to possible local actions, probably act on extrapineal target tissues.

In serotonergic cone-like photoreceptors of most lower vertebrates DCV have been observed. As in CP their functional significance is still unknown.

Although the photoperiodic information is supposed to be the principal input, it was hypothesized that ICP could transduce other inputs received either via efferent nerve fibers or by a hormonal type of action (Falcon, 1984).

2. Modified photoreceptor cells (one or several types ?) (Fig. 1)

a. Modified photoreceptor cells (= MP)

Well represented in chelonians, lacertilians and birds, they have been previously described and termed "rudimentary photoreceptor cells" (Collin and Oksche, 1981). Such cells were also found in the pike (Falcon, 1984). In the brook lamprey they are relatively scarce (Meiniel, 1981).

In the retina, as well as in the pineal organ of submammalian vertebrates, basic functional events take place in the outer segment and the synaptic pedicle of photoreceptor cells. Such events have been particularly well studied in retinal rods. Light causes a conformational change in rhodopsin and this, through the action of a second messenger, results in the closing of Na^+ channels in the plasma membrane, thereby hyperpolarizing the cell and decreasing its output of neurotransmitter. The signal is passed via a chain of neurons to the brain. If the term "photoreceptor cell" is interpreted in the conventional sense, in analogy to the rods, some CRL of the pineal organ cannot be regarded as conventional photoreceptors.

Between CP/ICP and MP there are two major differences which concern the polar cellular structures :

(i) CP and ICP show ribbon synapses serving, under particular conditions, the delivery of signals to other neurons afferent to the brain. On the contrary, in MP, the basal pedicle of originally synaptic character becomes independent of neurons and projects towards the external surface of the parenchyma displaying secretory polar terminals (Collin, 1969, 1971, 1977 ; Collin and Oksche, 1981 ; Collin et al., 1984). This "new" structural situation, as well as the results from electrophysiological studies (ref. in Collin et al., 1984), speak in MP against the presence of a sensory capacity (in the classical sense) for direct nervous responses to light.

(ii) The structure of the outer segment of MP has been considered to be rudimentary, in comparison to that of CP and ICP. However, recent data (for references, see Collin et al., 1984 ; Oksche, 1984) suggest that MP might still represent a functional light-sensitive unit, although this remains to be definitively established. The electrophysiological studies of Falcon and Meissl (1981) shows that MP of the pike are photosensitive.

The coexistence of several secretory products (indoles and "peptides") has been established in lizards and in a more detailed study in the avian material (ref. in Collin, 1979, 1981 ; Collin et al., 1984). In all these species, MP are indolergic and "peptidergic" cells (see above). Especially in birds, it was shown from numerous experiments that the contents of endogenous indoles or the in-vivo and in-vitro release of melatonin, and also enzymatic activities of indole metabolism undergo striking day-night changes in response to photoperiodic information (Ralph, 1976 ; Takahashi et al., 1980).

Together these data enable to propose a hypothesis according to which MP convert the photoperiodic information into non-neural responses (probably neurohormonal responses). The neurohormonal responses are mediated by active indoles (5-methoxyindoles), as has been shown in the pike, in the lizard, the parakeet, and the pigeon (see above). In sauropsids, these responses might also have a peptidic character, although it is still not established whether the protein secretion is identical with a peptidic neurohormone(s) (Section II). Accepting the fact that, in MP, one or more 5-methoxyindole(s) correspond to neurohormones, it may be suggested that MP represent another group of photoneuroendocrine cells.

The mechanisms of translation taking place in MP are of a particular interest (Collin et al., 1984). The pineal, an important component of the avian biological clock is, at least in some birds, considered as an oscillator although not self-sustained (e.g., in the chicken), since its rhythmicity may damp after 2-4 cycles in D/D (ref. in Menaker and Binkley, 1981). The chicken pineal contains multiple oscillators (for references see Cassone and Menaker, 1983 ; Collin et al., 1984).

Considering that indolergic MP might be photosensitive, in comparison with the other cell types of the avian pineal (CIL and rare neurons), they appear as the best candidates for a capacity as pacemakers. In other words, MP, at least in some species, may contain a circadian oscillator, synchronized by the light-dark cycle via a light-sensitive unit (in the outer segment ?) and determining the rhythms of synthesis and/or release of neuroendocrine messages (e.g., aMT). Cassonne and Menaker (1983) have shown that the orthosympathetic input from the superior cervical ganglia help to sustain the rhythmicity of the chicken pineal by the release of noradrenaline, presumably a result of an active regulation mechanism in unidentified circadian oscillators of the brain. It has been suggested that noradrenaline in vivo or in vitro inhibits aMT synthesis.

In summary, the cytophysiological, biochemical and physiological data implicate the following question : Do MP (or certain MP) represent circadian oscillators (see Collin et al., 1984, for more details) ?

b. **Highly modified photoreceptor-like cells (HMP)**

As already pointed out above, MP do not belong to the category of

conventional photoreceptors. In addition, polar structures (outer segments) which are a prerequisite of transduction may be extremely regressed or even absent in some CRL. Here, pinealocytes "sensu stricto" (= Pi : Collin, 1969, 1971 ; Oksche, 1971 ; Collin and Oksche, 1981) and HMP, can be concerned.

Such HMP were found in chelonians, lacertilians and birds (Collin, 1969, 1971, 1977 ; Collin and Oksche, 1981), and occasionally also in mammals (ref. in Collin, 1977). In ultrathin sections HMP may maintain an equivalent of the outer segment corresponding to a bulbous cilium (showing a 9 x 2 + 0 axonemal pattern), however apparently devoid of typical disks. The basal pedicle of HMP resembles that of MP. The rudimentary state or absence of a cilium raises the question of a direct photosensitivity, since the number of membranous photoreceptive molecules might be extremely reduced or absent in this type of outer segment. In reptiles and birds, the absence of an outer segment might depend on a sectioning artefact : ultrathin sections might display incompletely the structure of a normally reduced outer segment. Probable cyclic changes of rudimentary outer segments should also be considered (Section IV). Thus, complementary investigations must be carried out before coming to definitive conclusions with respect to the absence of a direct photosensitivity. In lizard, parakeet and pigeon, HMP, as defined above, appear as indolergic and peptidergic cells. In accord with the author's concept of MP, HMP are neuroendocrine.

3. Pinealocytes (sensu stricto) (Pi) (Fig. 1)

This cell type is predominant in mammals and ophidians ; it may also be found in certain regions of the pineal of birds and other reptilian orders.

In the rodent pineal, indole metabolism has been extensively studied. 5-methoxyindoles are synthesized, stored and released into the blood and CSF. At the cellular level, our knowledge on this metabolism is extremely restricted (Collin, 1979 ; Juillard et Collin, 1980 ; Juillard et al., 1984). In Pi, the absence of a direct response to light is generally accepted. Photoperiodic information is perceived by the lateral eyes and then transferred to the pineal via a multisynaptic neural pathway including an oscillator (ref. in Reiter, 1981 ; Cardinali, 1983). Although we failed to demonstrate that Pi are indolergic, it has been hypothesized that Pi translate rhythmic neural signals (by means of noradrenaline acting on a ß-receptor-cyclic AMP system) into neuroendocrine messages (5-methoxyindoles and possibly peptides) (Collin, 1979 ; Juillard and Collin, 1980). It must be noted that this hypothesis fits well into our knowledge of the phylogeny of CRL and the indolergic properties of some phylogenetic forerunners of Pi. In addition, the localization of hydroxyindole-O-methyltransferase in the cytoplasm of most bovine pinealocytes has recently been shown with the use of immunocytochemical methods (Kuwano et al., 1983).

C. CONCLUSIONS : CRL OF THE PINEAL ORGANS ARE CONNECTING LINKS BETWEEN ENVIRONMENT AND TARGET SITES

In conclusion, (i) the above - mentioned physiological and cytophysiological data and (ii) the fact that the circadian patterns of biosynthesis of indoles (e.g. 5-methoxyindoles) and neural responses (Meissl and Dodt, 1981) conform with photoperiod, strongly suggest that CRL translate

photoperiodic information into signals by means of informational molecules (unknown neurotransmitter(s), indoles and possibly also peptides = pinealins).

1. Direct transducers of photic information

CP, ICP and possibly MP respond to photic stimulation, but only CP and ICP send impulses to circumscribed regions of the brain, by means of (an) unidentified neurotransmitter(s) and via afferent neurons. In ICP of the pike, 5-methoxyindoles are synthesized and stored under manifestation of cyclic changes. The release of these pineal substances into the blood and CSF has not been verified in the lower vertebrates although it is very probable. It is strongly suggested that ICP translate photoperiodic stimuli into both neural and hormonal rhythmic outputs. Thus, CP and ICP represent at the cellular level direct transducer units.

MP may also function as transducer units, responding directly to light/dark stimulation, however only by means of an output of hormones (5-methoxyindoles and possibly peptides). In birds, MP might also be influenced by photic messages originating from the lateral eyes (Herbuté, 1983).

2. Indirect transducers of photic information

If rodent Pi indeed are indolergic (and possibly also peptidergic), cells which are indirectly influenced by photoperiod via a neuronal input represent "conveyors" of environmental stimuli. This conclusion is in accord with the postulates of Hoffmann (1981). Nevertheless, due to other integrative functions, Pi cannot be considered only as relay stations for photoperiodic mechanisms.

N.B. : It is still impossible to classify HMP either as transducers or "conveyors".

3. CRL : Multiple transducer/messenger cells ; circadian cellular clocks ?

On the basis of the above comments, it is tempting to make some speculations.

It must be noted that the photoperiodic information transmitted via the pineal organ, by means of informational molecules, appears to be complex, due to the plurality of CRL in a given pineal (Collin and Oksche, 1981) (Fig. 1). CRL are multitransducers since they respond primarily to the photoperiod and probably secondarily also to other environmental (Vivien-Roels, 1983) and hormonal (Cardinali, 1983) signals. During the course of vertebrate evolution two types of transduction of environmental light signals appear to be established in CRL, i.e., direct and indirect (= neural ; Pi, as defined above, might represent neuroendocrine transducers : Cardinali, 1983). Concerning the direct type of transduction it cannot be definitively excluded that photosensitive molecules (different from photopigments of outer segments ; cf. Hartwig and Oksche, 1982) may operate in some CRL (e.g. HMP ?). A photoreceptor cell might also trigger CRL which have lost their direct photosensitivity (= indirect transduction).

CRL can also be regarded as multimessenger cells (output of different types of informational molecules). Messengers might act at close range and/or at distance. The advantages of this type of operation are still not obvious.

aMT synthesis and release in the mammalian pineal gland are under the

control of a photoreceptor (lateral eyes) and a circadian oscillator (suprachiasmatic nuclei). On the contrary, the pineal organs of some sauropsids display simultaneously photoreceptors (CRL) and multiple oscillators capable of aMT synthesis and release (CRL) (see also Menaker and Wissner, 1983). It is hypothesized that some CRL (e.g. MP) might represent cellular clocks.

IV. THE CONCEPT OF CRL : OUTLOOK

In life sciences models or concepts are to be regularly re-evaluated, since after some time the data on which they are based appear to be incomplete.

The concept of "cells of the receptor line" (CRL) was introduced and developed first independently and later in cooperation by Collin (1969, 1971, 1977), Oksche (1971), Collin and Oksche (1981).

Ontogenetic and phylogenetic considerations, based on cellular homologies, led to propose that pineal cells of the "photoreceptor" type can be arranged in a series or line. Clear-cut structural remodelling of these cells is closely linked to functional recycling. Although this was never strongly emphasized, the idea of a "line", in our opinion, must be considered in a somewhat wider sense. It would be indeed an error to reject a priori the possibility of the existence of several lines (or sub-lines) of photoreceptors and their homologues. Nevertheless, from the pineal organ of recent vertebrates it is difficult to postulate whether in the ancient vertebrates one or several types of primordial photoreceptor cells were the matrix of one or several lines of "photoreceptor" cells. The differentiation of several types of typical photoreceptors and their corresponding derivatives - from a common ancestral photoreceptor cell - might also be a plausible developmental pattern that occurred during the course of vertebrate evolution.

Irrespective of the numerous data accumulated during the last 15 years, Section III leaves many questions open to discussion. The synopsis of CRL in this Section and Figure 1 does not present a new classification. It only indicates, in connection with the respective comments, some of the new alleys of research.

Keeping these ideas in mind and prior to trying to establish a more complete model, a great effort is required to increase our knowledge on the structural-functional properties of the CRL. It is necessary to fill gaps concerning the chemical nature and cellular sites of different types of molecules, e.g. photopigments, neurotransmitters, hypothetical peptidic neurohormones... Data on indole metabolism are far from being complete. Our knowledge concerning (i) homologies among the different derivatives of the diencephalic roof, and (ii) cell differentiation during ontogenesis, remains to be increased. In addition, detailed comparisons between CRL and other diencephalic ocular and extraocular photoreceptor cells which in their entirety might have a common origin (Hartwig and Oksche, 1982) would be of a great value for establishing the different lines of diencephalic photoreceptors.

Structural and molecular changes (e.g., during the 24h-cycle and the course of a year) must be examined in detail. Conspicuous cyclic structural changes have been shown mainly in photoreceptor cells and pinealocytes. For example, a vivid process of the renewal of photoreceptor outer segments takes place in lower vertebrates (cf. Collin and Oksche, 1981). Recent data obtained by Hartwig (1984) give clear indication of this renewal in the frog and suggest that two or more morphologically defined members of the receptor line might correspond to different

physiological states of a single cell type. In other words, CP (or ICP) and MP may represent only cyclic changes of one and the same cellular type. Unfortunately, the cellular nycthemeral changes of e.g. exportable indole molecules and ultrastructures of synaptic pedicles were as yet not studied in the frog. In the context with his findings, Hartwig (1984) mentions that the notion of rudimentation during evolution, as proposed by Collin and Oksche, should be re-evaluated. Obviously it cannot be excluded that reduced and typical outer segments might be subjected to a renewal during a 24h-cycle. According to our present opinion, the great variability and the rudimentation of the outer segments in vertebrates may correspond not only to cyclic phases but also to different genetically-controled stages of differentiation during ontogeny and phylogeny (Collin and Oksche, 1981 ; Collin et al., 1984). In favor of this viewpoint appears to be the fact that, in lower vertebrates, Pi are rare and may even be absent* but in mammals, on the contrary, typical CP or ICP were never found. In other words, comparing remote classes of vertebrates, I have some difficulties to imagine that during a 24h-cycle CP or ICP are capable to transform into Pi (or Pi into CP or ICP), if for exemple fishes and mammals are compared. Are desynchronized patterns of membrane renewal of outer segments sufficient to explain why in the wall lizard investigated in June, the basically different CP (or ICP ?) and MP are found together at the end of the photophase (Collin, 1969) ?

It must be emphasized that, in Section III, only photoperiodic information among other stimuli has been considered. As proved by numerous authors, other environmental, hormonal and humoral inputs influence the function of the pineal organ. Although, for instance, typical structural differentiations for transduction of other environmental stimuli were not clearly demonstrated in CRL, it cannot be predicted how the transduction mechanisms of this type might influence the classification of the components of the receptor line.

The presentation of CRL in Figure 1 can only be tentative since numerous crucial parameters are still missing. The distinction between cone-like photoreceptors (CP and ICP) in Anamnia and reptiles is based mainly on the presence or absence of HT. In contrast, the discrimination between MP and VMP has been focussed only on structure-function relationship in the outer segment and might be provisional. Falcon (1984) also mentioned that, in the pike, his separation of ICP and MP might be preliminary since the photopigments of both cell types (Falcon and Meissl, 1981) have not been identified chemically and localized topographically. This means, for instance, that ICP might be subdivided into two categories of cells, each endowed with an individual photopigment.

Thus, new subdivisions or fusions of the present schematic line of cell types (Figure 1) remain disputable.

Finally there is a number of reasons indicating that a definitive classification as well as a subdivision of the original cell line sensu lato into lines sensu stricto or sub-lines will require additional systematic experimental work.

As mentioned by Collin and Oksche (1981 ; see also previous reports), I believe that the differentiation of CRL parallels the phylogeny of vertebrates and that a plurality of CRL in a given pineal organ exists.

Although the pineal organ was often considered as a relatively simple structure (e.g., in comparison to that of the retina of the lateral eyes),

*Cole and Youson (1982) are not definitively convinced that pinealocytes occur in the lamprey, as has been previously proposed by Meiniel (1981).

it appears to be very complex at the cellular level. The term "pinealocyte", used by some authors to refer to either CRL or to pineal cells, does not reflect the structural and functional heterogeneity of the pineal cell types.

I have attempted to comment on some of the exciting aspects arising from the increasing knowledge of pineal messengers and properties of CRL. Much remains to be discovered by use of the available and future tools of cell biology and physiology.

REFERENCES

BALEMANS M.G.M. (1981). Indole metabolism in the pineal gland, the Harderian gland and the retina of mammals. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, pp. 261-279.

BALEMANS M., COLLIN J.P., LEGERSTEE W.C. and JUILLARD M.T. (1981). Preliminary investigations on the circadian rhythmicity of the methylation of 5-hydroxyindoles in the pineal gland of the parakeet. Biol. Cell., 42, 167-172.

BALEMANS M., JUILLARD M.T., Van BENTHEM J. et COLLIN J.P. (1983). Rétention des indoles-(^{3}H) dans l'organe pineal d'Oiseau (Melopsittacus undulatus, Shaw) au cours de sa préparation pour l'étude radioautographique. Biol. Cell., 49, 257-266.

CARDINALI D.P. (1983). Molecular mechanisms of neuroendocrine integration in the central nervous system : an approach through the study of the pineal gland and its innervating sympathetic pathway. Psychoneuroendocrinology, 8, 3-30.

CASSONE V.M. and MENAKER M. (1983). Sympathetic regulation of chicken pineal rhythms. Brain Res., 272, 311-317.

COLE W.C. and YOUSON J.H. (1982). Morphology of the pineal complex of the anadromous sea lamprey, Petromyzon marinus L. Amer. J. Anat., 165, 131-163.

COLLIN J.P. (1969). Contribution à l'étude de l'organe pinéal. De l'épiphyse sensorielle à la glande pinéale : modalités de transformation et implications fonctionnelles. Ann. Stat. Biol. Besse-en-Chandesse, suppl. 1, 1-359.

COLLIN J.P. (1971). Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In : The pineal gland (A Ciba Foundation Symposium) (eds. G.E.W. WOLSTENHOLME and J. KNIGHT), Churchill Livingstone, Edinburgh, London, pp. 79-120.

COLLIN J.P. (1977). La rudimentation des photorécepteurs dans l'organe pinéal des Vertébrés. In : Mécanismes de la rudimentation des organes chez les embryons de vertébrés (ed. A. RAYNAUD), Editions du CNRS, n° 266, Paris, pp. 393-407.

COLLIN J.P. (1979). Recent advances in pineal cytochemistry. Evidence of the production of indoleamines and proteinaceous substances by rudimentary photoreceptor cells and pinealocytes of Amniota. Prog. Brain Res., 52, 271-296.

COLLIN J.P. (1981). New data and vistas on the mechanisms of secretion of proteins and indoles in the mammalian pinealocyte and its phylogenetic precursors ; the pinealin hypothesis and preliminary comments on membrane traffic. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 187-210.

COLLIN J.P. and OKSCHE A. (1981). Structural and functional relationships in the nonmammalian pineal gland. In : The pineal gland, vol. I : Anatomy and Biochemistry (ed. R.J. REITER), CRC Press, Inc. Boca Raton, Florida, pp. 27-67.

COLLIN J.P., BALEMANS M., JUILLARD M.T., LEGERSTEE W.C., Van BENTHEM J. and VOISIN P. (1982). Indole metabolism at the cellular level : an in vivo combined radiobiochemical and high-resolution autoradiographic study in the avian pineal, with special reference to melatonin/5-methoxytryptophol. Biol. Cell., 44, 25-34.

COLLIN J.P., JUILLARD M.T. and VOISIN P. (1984). Circadian information and messages in the modified photoreceptor cells of the avian pineal organ : retrospect and prospect. Ophthalmic Res., 16, 107-113.

de VLAMING V. and OLCESE J. (1981). The pineal and reproduction in fish, amphibians, and reptiles. In : The pineal gland, Vol. II : Reproductive effects (ed. R.J. REITER), CRC Press, Inc. Boca Raton, Florida, pp. 1-29.

FALCON J., JUILLARD M.T. et COLLIN J.P. (1980). L'organe pinéal du Brochet (Esox lucius, L.). V. Etude radioautographique de l'incorporation in vivo et in vitro de précurseurs indoliques. Reprod. Nutr. Dévelop., 20, 991-1010.

FALCON J. and MEISSL H. (1981). The photosensory function of the pineal organ of the pike (Esox lucius, L.). Correlation between structure and function. J. Comp. Physiol., 144, 127-137.

FALCON J. (1984). Identification et propriétés des cellules photoneuroendocrines de l'organe pinéal. Thèse de Doctorat d'Etat, n° 384, Univ. Poitiers, France.

FALCON J., GEFFARD M., JUILLARD M.T., STEINBUSCH H.W.M., SEGUELA P. and COLLIN J.P. (1984). Immunocytochemical localization and circadian variations of serotonin and N-acetyl-serotonin in photoreceptor cells. Light and electron microscopic study in the teleost pineal organ. J. Histochem. Cytochem.,32, 486-492.

HARTWIG H.G. and REINHOLD C. (1981). Microspectrofluorometry of biogenic monoamines in pineal systems. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 237-246.

HARTWIG H.G. and OKSCHE A. (1982). Neurobiological aspects of extraretinal photoreceptive systems : structure and function. Experientia, 38, 991-996.

HARTWIG H.G. (1984). Cyclic renewal of whole pineal photoreceptor outer segments. Ophthalmic Res., 16, 102-106.

HERBUTE S. (1983). Epithalamus et régulations neuroendocriniennes. Thèse de Doctorat d'Etat, Univ. Montpellier, France.

HOFFMANN K. (1981). Photoperiodic function of the mammalian pineal organ. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, pp. 123-138.

JUILLARD M.T., HARTWIG H.G. and COLLIN J.P. (1977). The avian pineal organ. Distribution of endogenous monoamines ; a fluorescence microscopic, microspectrofluorimetric and pharmacological study in the parakeet. J. Neural Transmission, 40, 269-287.

JUILLARD M.T. and COLLIN J.P. (1979). Membranous sites of oxidative deamination : a comparison between ultracytochemical and radioautographic studies in the pineal organ of the wall lizard and the parakeet. Biol. Cell., 36, 29-36.

JUILLARD M.T. and COLLIN J.P. (1980). Pools of serotonin in the pineal gland of the mouse : the mammalian pinealocyte as a component of the diffuse neuroendocrine system. Cell Tissue Res., 213, 273-291.

JUILLARD M.T., BALEMANS M., COLLIN J.P., LEGERSTEE W.C. and VAN BENTHEM J. (1983). An in vitro combined radiobiochemical and high-resolution autoradiographic study predicating evidence for indolergic cells in the avian pineal organ. Biol. Cell., 47, 365-378.

JUILLARD M.T., COLLIN J.P., BALEMANS M.G.M. and QUEAU A. (1984). In vitro uptake and metabolism of (^{3}H)-5-hydroxytryptamine in the pineal glands of the rabbit, rat and hamster. A comparative study with the use of autoradiography, chromatography and liquid-scintillation counting. Cell Tissue Res., 235, 539-549.

KUWANO R., IWANAGA T., NAKAJIMA T., MASUDA T. and TAKAHASHI Y. (1983). Immunocytochemical demonstration of hydroxyindole O-methyltransferase (HIOMT), neuron-specific enolase (NSE) and S-100 protein in the bovine pineal gland. Brain Res., 274, 171-175.

MATSUURA T., KAWATA M., YAMADA H., KOJIMA M. and SANO Y. (1983). Immunohistochemical studies on the peptidergic nerve fibers in the pineal organ of the dog. Arch. histol. jap., 46, 373-379.

MATSUURA T. and SANO Y. (1983). Distribution of monoamine-containing nerve fibers in the pineal organ of untreated and sympathectomized dogs. Cell Tissue Res., 234, 519-531.

McNULTY J.A. (1984). Functional morphology of the pineal complex in cyclostomes, elasmobranchs and bony fishes. Pineal Res. Rev., 2, 1-40.

MEINIEL A. (1981). New aspects of the phylogenetic evolution of sensory cell lines in the vertebrate pineal complex. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 27-47.

MEISSL H. and DODT E. (1981). Comparative physiology of pineal photoreceptor organs. In : The pineal organ : Photobiology - Biochronometry - Endocrinology (eds. A. OKSCHE and P. PEVET), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 61-80.

MENAKER M., BINKLEY S. (1981). Neural and endocrine control of circadian rhythms in the vertebrates. In : Handbook of behavioral neurobiology, Vol. 4, (ed. J. ASCHOFF), Plenum Publ. Corp., New York, pp. 243-255.

MENAKER M. and WISNER S. (1983). Temperature-compensated circadian clock in the pineal of Anolis. Proc. Natl. Acad. Sci. USA, 80, 6119-6121.

MØLLER M. and van VEEN Th. (1981). Fluorescence histochemistry of the pineal gland. In : The pineal gland, Vol. I : Anatomy and Biochemistry (ed. R.J. REITER), CRC Press, Inc. Boca Raton, Florida, pp. 69-93.

NURNBERGER F. and KORF H.-W. (1981). Oxytocin- and vasopressin-immunoreactive nerve fibers in the pineal gland of the hedgehog, Erinaceus europaeus L. Cell Tissue Res., 220, 87-97.

OKSCHE A. (1971). Sensory and glandular elements of the pineal organ. In : The pineal gland (A Ciba Foundation Symposium) (eds. G.E.W. WOLSTENHOLME and J. KNIGHT), Churchill Livingstone, Edinburgh, London, pp. 127-146.

OKSCHE A. (1984). Evolution of the pineal complex : correlation of structure and function. Ophthalmic Res., 16, 88-95.

PEVET P. (1983). The different classes of proteic and peptidic substances present in the pineal gland. In : The pineal gland and its endocrine role (eds. J. AXELROD, P. FRASCHINI and G.P. VELO), Plenum Publ. Corp., New-York, pp. 113-149.

PIEKUT D.T. and KNIGGE K.M. (1982). Immunocytochemical analysis of the rat pineal gland using antisera generated against analogs of luteinizing hormone-releasing hormone (LHRH). J. Histochem. Cytochem., 30, 106-110.

PRECHEL M.M., AUDHYA T.K. and SCHLESINGER D.H. (1983). A seasonal variation in arginine vasotocin immunoactivity in rat pineal glands. Endocrinology, 112, 1474-1478.

RALPH C.L. (1976). Correlations of melatonin content in pineal gland, blood, and brain of some birds and mammals. Amer. Zool., 16, 35-43.

RALPH C.L., FIRTH B.T. and TURNER J.S. (1979). The role of the pineal body in ectotherm thermoregulation. Amer. Zool., 19, 273-293.

RALPH C.L. (1983). Evolution of pineal control of endocrine function in lower vertebrates. Amer. Zool., 23, 597-605.

REITER R.J. (1981). The pineal gland. Anatomy and Biochemistry. Vol. 1, CRC Press, Inc. Boca Raton, Florida, 1-320.

REITER R.J. (1983). The pineal gland : an intermediary between the environment and the endocrine system. Psychoneuroendocrinology, 8, 31-40.

RIX E., HACKENTHAL E., HILGENFELDT U. and TAUGNER R. (1981). Neuropeptides in the pineal gland ? A criticial immunocytochemical study. Histochemistry, 72, 33-38.

TAKAHASHI J.S., HAMM H. and MENAKER M. (1980). Circadian rhythms of melatonin release from individual superfused chicken pineal glands in vitro. Proc. Natl. Acad. Sci. USA, 77, 2319-2322.

UECK M. (1979). Innervation of the vertebrate pineal. Prog. Brain Res., 52, 45-87.

VEEN Th. van, EKSTROM P. and BORG B. (1980). The pineal complex of the three-spined stickleback, Gasterosteus aculeatus L. A light-, electron microscopic and fluorescence histochemical investigation. Cell Tissue Res., 209, 11-28.

VIVIEN-ROELS B. (1983). The pineal gland and the integration of environmental information : possible role of hydroxy- and methoxyindoles. Molecular Physiol., 4, 331-345.

VOISIN P., GEFFARD M., DELAAGE M. et COLLIN J.P. (1982). Mélatonine dans l'organe pinéal, la rétine et le plasma. Etude immunologique chez le Pigeon. Reprod. Nutr. Dévelop., 22, 959-971.

VOISIN P., JUILLARD M.T. and COLLIN J.P. (1983). Indole metabolism in the pineal organ of the pigeon with special reference to melatonin-synthesizing cells. In vitro study combining thin layer chromatography, autoradiography and pharmacological treatment. Cell Tissue Res., 230, 155-169.

The Pineal Gland
Current State of Pineal Research
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PINEAL PEPTIDES: A CURRENT STATUS

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INTRODUCTION

In biological research, the efforts to reconcile conflicting data has often resulted in a better understanding of hitherto unclear, conflicting findings. Sometimes, relationships between conflicting but reproducible results were obtained. Such efforts, with respect to the presence and significance of pineal peptidic- and proteic substances, have been made during nearly two decades.
At the ultrastructural level, processes implicated in the synthesis and release of peptidic/proteic compounds have been demonstrated in pineal specific cells by different authors. (For details see Ariëns Kappers 1979; Collin 1979, 1981; Collin and Oksche 1981; Pévet 1979, 1981a,b, 1983).
However, up till now, the applied classical methods for peptide/protein purification have failed to yield sufficiently "large" quantities of pure pineal peptides/proteins for structure elucidation and detailed biological experiments. Moreover, a simple and specific bioassay, indispensable for the isolation of biologically active pineal compounds, has still to be found.
Different observations however, support the presence of pineal peptidic and proteic substances related to a definite biological activity. (See for details Benson 1979; Ebels and Benson 1978; Benson and Ebels 1981; Benson et al. 1983).
However, the conclusion of Vaughan (1981) is still true : "That after two decades of research can be concluded that the nona-peptide [8-arginine]-vasopressin (AVP) directly or indirectly affects the reproductive system of all classes of vertebrates, but that there is a considerable controversy whether an antigonadotropic peptide of the pineal is AVP, [8-arginine]-vasotocin (AVT), oxytocin (OT), E5 or another unidentified peptide".
It is the aim of this article to give a survey of the results of experiments carried out at different institutes in the last years, with respect to the above described problems. According to Pévet (1983) the peptidic and proteic substances present in the pineal can be divided into three classes:

1) compounds synthesized in organs other than the pineal, but found there because they are present in cell elements such as nerve axons, which connect the gland to different brain structures.
2) substances synthesized in organs other than the pineal, with a secondary uptake by the gland from the general circulation.
3) peptidic/proteic compounds which are synthesized in the pineal gland by specific pineal cell-bodies.

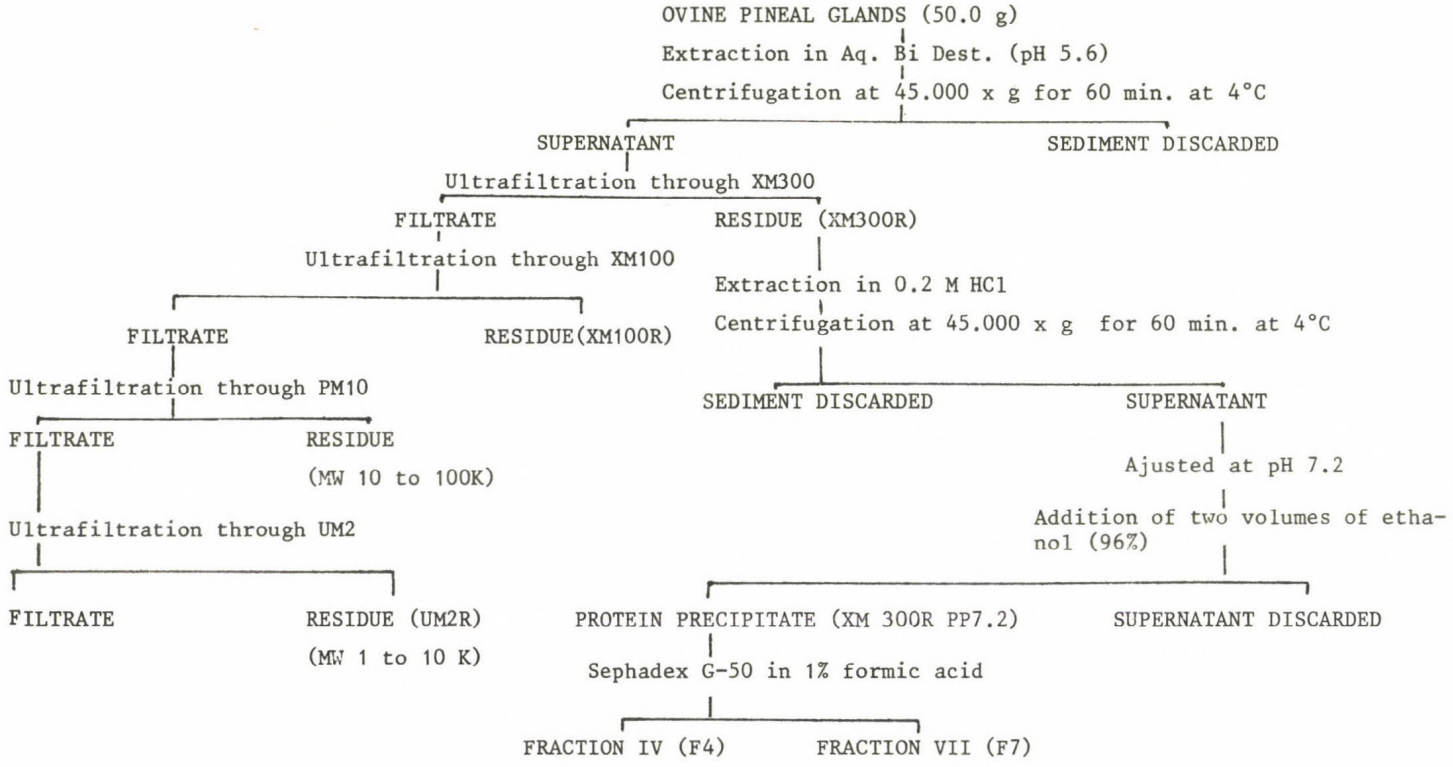
In this article we will follow this classification, but it may be that in

the future, the whole pineal peptide/protein biosynthesis is more complicated than we now assume and that peptides (which are now classified in one of the classes) may belong to another group.

SURVEY OF THE THUS FAR OBTAINED RESULTS

A. Compounds Belonging to Class 1

Regarding the above described classification, the neurohypophyseal principles as AVP, AVT, OT and neurophysin (NP) belong to class one. The results achieved in the last years with ovine pineal extracts will be discussed. In our institute, different ovine pineal preparations were made (see Scheme 1) in the last years. All the prepared fractions (see Scheme 1) were checked for the presence of neurohypophyseal principles by radioimmuno-assays (RIA) with antisera raised against AVP, OT (see Dogterom et al. 1977) and with an antiserum directed against the C-terminal tripeptide: Pro-Arg-Gly (NH_2) of AVP (Czernichow et al. 1974). Using these RIA's, it was found that the fraction XM300R PP7.2 contains AVP-like material, while the UM2R ultrafiltration-fraction contains mainly OT-like compound(s). The fraction XM300R PP7.2 was applied to a Sephadex G-50 column (98 x 1.6 cm) equilibrated and eluted with 1% formic acid (v/v). The subfractions of the Sephadex G-50 column (Fig. 1) were measured by RIA's for cross-reactivity with Pro-Arg-Gly(NH_2) and AVP. A single peak of AVP-like immunoreactivity, eluted between 158 ml and 180 ml, could be determined and a peak of oxytocin-like immunoreactivity eluted between 129 ml and 158 ml. These elution volumes correspond to a molecular weight (MW) range of 1 to 2 K. In the radioimmunological assays, the dilution curves of these pineal fractions showed a parallelism with the AVP, OT and Pro-Arg-Gly(NH_2) standard curves.
The UM2R-fraction (Scheme 1) was also applied to columns of Sephadex G-50 (98 x 1.6 cm) and of Sephadex G-25 (100 x 0.9 cm) equilibrated and eluted with 1% formic acid (v/v). The subfractions were measured by RIA for AVP-like and OT-like immunoreactivity. This time mainly oxytocin-like activity could be detected.
Reverse phase high performance liquid chromatography (RP-HPLC) of the XM300R PP7.2 Sephadex G-50 F7 revealed that the AVP-like immunoreactivity of that fraction was eluted in a position identical to that of synthetic AVP.
The UM2R Sephadex G-25 fraction, containing radioimmunological OT, could be separated on the same HPLC-column. Here the pineal oxytocin was eluted in the same position as synthetic OT, using the methanol/water mixture as for AVP.
Comparable experiments were carried out by us with the bovine pineal fraction E5, received from Dr. P. Pévet, The Netherlands Institute for Brain Research, Amsterdam, The Netherlands, and prepared by Dr. C. Neacşu, Bucharest, Romania.
In HPLC-experiments, using subsequently different methanol/water mixtures, all the immunoreactive X-Pro-Arg-Gly(NH_2) peptides were located in the same region where synthetic AVP was eluted. In one methanol/water mixture, a peak was detected at the same place as where synthetic AVT was eluted. However, when another solvent mixture of methanol/water was used, it was possible to separate the peak of synthetic AVT and the peak present in ovine and bovine pineal extracts. When these last peaks were submitted to an amino acid analysis no amino acids were detectable.
From all these experiments was concluded that most probably, AVP and OT are present in ovine and bovine pineal extracts and can be isolated and identified with the methods just described. However, no evidence was



Scheme 1. Extraction- and purification scheme for neurohormone-like principles from ovine pineal glands.

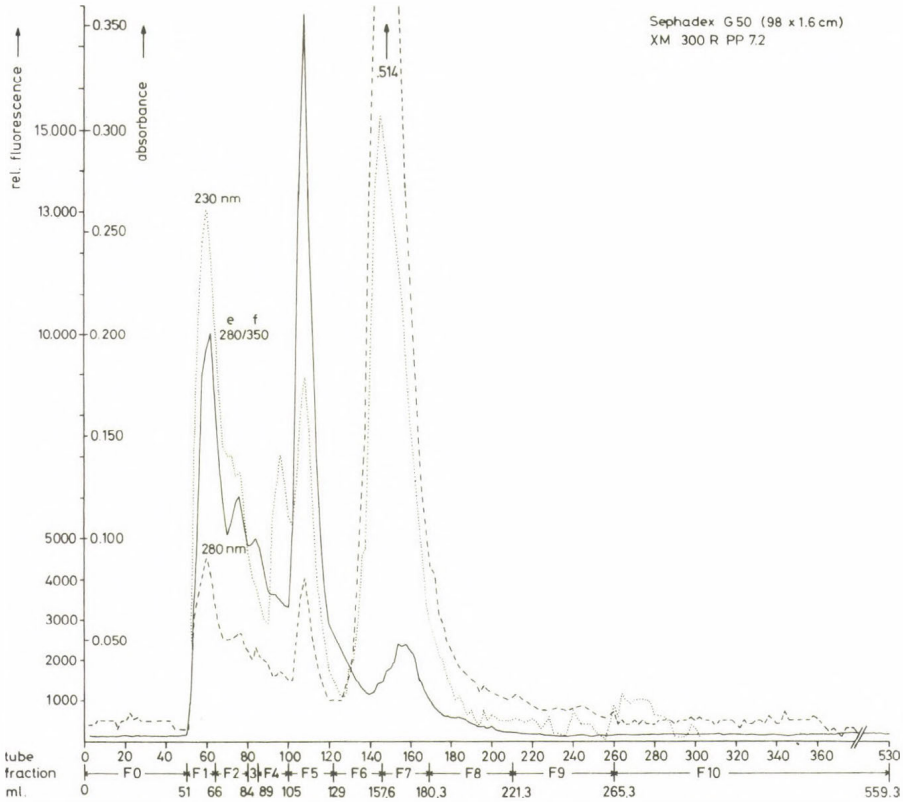


Figure 1. Gel filtration of the ovine pineal fraction XM300R PP7.2 (see Scheme 1) on Sephadex G-50 superfine. Column: 1.6 x 98 cm; equilibrated and eluted with 1% formic acid; flowrate: 7.14 ml. $cm^{-2}.h^{-1}$; fraction size: 1 ml.

····· absorbance at 230 nm

---------- absorbance at 280 nm

———— excitation maximum (e) at 280 nm
emission maximum (f) at 350 nm

obtained for the presence of AVT, nor for the presence of Pro-Arg-Gly(NH_2) peptides other than AVP in pineal extracts. Experimental details will be published in the near future e.g. Thesis Noteborn (in preparation).
The study of pineal neurophysin was carried out with the antiserum AS 210, generated in rabbit against bovine pituitary AVP-NP. This particular antiserum was used because it cross-reacts with pituitary neurophysins of many other mammals such as man, rabbit, rat, mouse and sheep.(See for details Reinharz and Vallotton 1977; Reinharz et al. 1981). When aqueous ovine pineal extracts are ultrafiltrated through Amicon Diaflo-membranes, two ovine pineal fractions could be obtained which contained immunoreactive neurophysin (IMR-NP) namely XM300R PP7.2 and PM10R (see Scheme 1). From 50 g of wet ovine pineal tissue 552 µg of IMR-NP were obtained.
It was found that about 5% of these IMR-NP are eluted from three different Sephadex columns with an elution volume corresponding with molecules with a MW > 10.000, between bovine serum albumin and pituitary neurophysin (MW = 10.000). This last pineal neurophysin shares immunological and physico-chemical properties with highly purified bovine pituitary neurophysin used as a reference. From the results obtained with gelfiltration and affinity chromatography on [8-lysine]-vasopressin Sepharose, it was concluded that ovine pineal glands may contain a neurophysin precursor molecule in addition to the neurophysins of MW = 10.000. For more experimental details see Reinharz et al. (submitted for publication and Thesis Noteborn in preparation).

B. Compounds Belonging to Class 2

To the second class of pineal peptides the hypothalamic and (adeno)hypophyseal hormones are now considered to belong. In the last years, different authors have demonstrated that with radioimmuno-assays these hormones can be detected in pineal tissue (Pévet 1983). An important aspect of pineal physiology is that these observations suppose the existence of a feed-back system, operating between the hypothalamus, the hypophysis and the pineal, which would implicate the occurence of hypophyseal hormones in the pineal. That would mean that the adenohypophyseal hormones, luteinizing hormone(LH), follicle stimulating hormone (FSH), growth hormone (GH) and prolactin (Prl) should be chemically detectable in the pineal organ. Noteborn et al. (in preparation) have tried to isolate and characterize adenohypophyseal hormones with a method published by Ellis (1961) in order to isolate and identify the hormones LH, FSH and GH from pineal extracts. The results of these experiments revealed that it is possible to isolate and characterize different molecular forms of immunoreactive LH-like peptides (IMR-LH) from ovine pineal glands, using an 0.1 M ammoniumsulfate (pH 4.0) extract by application of the method developed by Ellis (1961).
The majority of pineal gland IMR-LH behaves identically to hypophyseal luteinizing hormone (LH) with an apparent MW of 21.000 daltons and a potency of 1.57 times NIAMD rat-LH RP-1 by rat Leydig-cell stereoidogenesis-assay. A small amount of pineal gland IMR-LH was found chromatographically with a MW of about 52.000 daltons, however without biological potency. These results confirm the observation of Reiss et al. (1963), but in our opinion the stimulatory principle occuring in the pineal gland is similar to the gonadotropic hormones (LH/FSH) of the anterior pituitary. Further studies have to be carried out in order to investigate whether an how LH-like peptides in the pineal gland are involved in reproductive phenomena. For more experimental details see Noteborn et al. (in preparation).
It was also possible to isolate and partially characterize an immunoreactive prolactin-like peptide (IMR-Prl) from frozen ovine pineal glands

by application of a slightly modified method developed by Jiang and Wilhelmi (1965), which differs on essential points from the method used for the isolation of LH/FSH. The results of these experiments have shown that the majority of ovine pineal IMR-Prl behaved identically to that of hypophyseal Prl in HPLC and polyacrylamide gelelectrophoresis. Chemical studies on our isolated ovine pineal prolactin suggest however, a heterogeneity comparable to pituitary prolactin preparations as described by Hwang et al. (1972).
The isolated ovine pineal prolactin is radioimmunologically active and displaces ^{125}I-Prl in a radioreceptor-assay using pregnant rat liver Prl-receptor preparations. The present data suggest that probably, native hypophyseal Prl occurs in the ovine pineal gland, which may be responsible for some stimulatory effects in bioassays. Here also further studies are needed to investigate whether and how Prl-like peptides in the pineal gland are involved in reproductive phenomena. Consequently, the possibility that the presence of such hormones within the pineal could be due to receptor activity has to be taken into consideration (see also Dubé et al. 1980).

C. Compounds Belonging to Class 3

The third group of pineal peptides/proteins is probably the most interesting group for pinealogists and endocrinologists.
Over about a period of twenty years, different research groups have tried to isolate, purify and identify pineal specific compound(s) with antigonadotropic activity, using different bioassays. For a survey of the literature see Ebels and Benson (1978), Benson and Ebels (1981) and Ebels (1983). This antigonadotropic activity e.g. in a compensatory ovarian hypertrophy (COH) assay after unilateral ovariectomy, is sensitive to trypsin- and chymotrypsin-digestion and is therefore thought to be a peptide or contain a peptide-moiety which is important for the biological activity. When cerebral cortex is treated in the same way as pineal glands, this antigonadotropin (PAG) is rather pineal-specific. Last year Benson et al. (1983) published new data about this PAG, using new bioassays and modern separation techniques e.g. HPLC.
In the future, it is possible that these experiments could lead to a better understanding of this pineal peptidic compound in relation to hypothalamic activity and on the biosynthesis and release of some adenohypophyseal hormones e.g. LH and Prl.
The results of these studies have shown, that a partially purified low MW pineal fraction augments the negative feed-back effects of testosterone on serum LH-levels in castrated mice. This fraction had no effect on pituitary LH-release in in vitro, but when injected into unanesthesized rats it caused a rapid and significant reduction in plasma LH- and Prl-levels. After injection of this bovine pineal fraction, a significant increase in the hypothalamic de novo dopamine synthesis using a deuterated tyrosine precursor was also observed. These observations suggest that effects on pituitary LH- and Prl-release may be mediated through the turnover of hypothalamic catecholamines.
When young adult female hamsters, acclimatized to long photoperiods (Light/Dark (L/D) = 14/10 h) are transferred to short photoperiods (L/D = 10/14 h) already after three weeks a highly significant increase in the turnover rates of hypothalamic dopamine is observed. The changes in dopamine turnover parallels the changes in the appearance of immunocytochemically-stained pituitary lactotrophs, using the peroxidase-antiperoxydase (PAP) technique for prolactin. Compared to long photoperiod controls, stainable Prl appears to be reduced between three and six weeks after short photoperiod-treatment (Benson, personal communications 1984).

The experimental results of Benson et al. (1983) and Benson (personal communications 1984) support the hypothesis that when seasonal breeders are transferred from a stimulatory to an inhibitory photoperiod, the pineal gland produces, via effects on hypothalamic catecholamine synthesis, an antigonadotropin which alters GrRH and Prl-inhibiting-factor-levels.

D. Effects of different synthetic compounds and hormones on the peptidic/proteic processes of the mammalian pineal

Several research groups have studied the influence of different synthetic compounds and hormones on the peptidic/proteic processes of the mammalian pineal in in vivo and in in vitro.
Karasek (1974) concluded from his experiments that the ultrastructure of the rat pineal gland, cultured in the presence of norepinephrine, dibutyryl cyclic adenosine 3',5'-monophosphate and adenohypophysis has many features in common, which can be interpreted as a morphological stimulation pattern of the pineal gland by the above mentioned compounds. The same author (Karasek et al. 1976) showed that after castration of adult male rats, the ultrastructure of pinealocytes was changed: an increased development of the rough endoplasmatic reticulum (RER) and the Golgi-apparatus as well as an increase in the number of lipid droplets and lysosomes were observed. The morphological changes after orchidectomy followed by LH-RH administration, were more pronounced compared with only castrated animals. These authors have put forward the working hypothesis that a negative feedback mechanism between the pineal and adenohypophysis may exist.
Karasek and Marek (1978) have studied the influence of gonadotropic hormones on the ultrastructure of rat pinealocytes. They concluded that after injection of human chorionic gonadotropin (HCG), a marked activation of pinealocytes was observed; e.g. proliferation of the granular endoplasmatic reticulum and Golgi-apparatus as well as an increase in number of dense core vesicles and mitochondria.
Karasek (1978) observed that after hypophysectomy, the ultrastructural pattern of pinealocytes showed a general diminished activity, indicated by a decrease in the granular endoplasmatic reticulum, lysosomes and lipid droplets. It is also stated in this paper, that after HCG-injections, the observed stimulation of the pinealocytes particularly concerns the cell organelles involved in protein synthesis. They (Karasek et al. 1982a) described that prolactin injections in rats stimulate the ependymal-like secretory process: granular endoplasmatic reticulum and vacuoles containing flocculent material. They also (Karasek et al. 1982b) gave a survey of dense-core vesicles in the mammalian pinealocytes and their relation to secretory processes, using different animals.
The conclusion from all the above described experiments must be that although the secretory process in the mammalian pinealocyte can be characterized morphologically, influenced by different synthetic compounds and by hormones, further investigations are necessary to elucidate the role of the peptide/protein synthesis in pineal metabolism and in gonadotropic hormone-regulation in general.
Haldar-Misra and Pévet (1982, 1983, a,b,c,d,e,) have also studied the influence of different compounds on the process of proteic/peptidic secretion in the mammalian pineal organ using organ cultures. The processes involved in the synthesis of peptidic/proteic agents have been identified by these authors at the ultrastructural level, characterized either by the formation of granular vesicles (GV) by the Golgi-saccules or by the formation of vacuoles containing flocculent material of moderate electron density by the cisternae of the RER.

Unfortunately the reported ultrastructural observations do not give an answer to the question, whether the two identified secretory processes involved in the synthesis/secretion of peptidic/proteic material are coupled or whether they function independently. It is necessary to precise further why the process characterized by the formation of GV is poorly developed in most of the mammalian species studied. For a survey of these processes in the pineal, see Collin (1979, 1981), Collin and Oksche (1981), Pévet (1979, 1981 a,b, 1983). Haldar-Misra and Pévet (1982) concluded from their studies that noradrenalin (NA) is implicated in the regulation of the production of the peptidic/proteic agents. However, it seems that the effect of this neurotransmitter is species-specific.

The influence of melatonin and other 5-methoxyindoles on the process of protein and/or peptide secretion was also studied by these authors. Melatonin in the absence of NA in the medium, induces the formation of GV by the Golgi-apparatus in the pinealocytes of the rat, but not in those of the golden Syrian hamster. In the presence of NA, melatonin provokes a decrease in the number of these vesicles in both species. For details see Haldar-Misra and Pévet (1983a).

All 5-methoxyindoles studied influence the number of GV in mice pinealocytes maintained in explant culture. 5-Methoxyindole-3-acetic acid in the presence or absence of NA increased the number of GV. 5-Methoxytryptamine, 5-methoxytryptophan, 5-methoxytryptophol and melatonin affect the process of protein/peptide secretion depending on the duration of application and the presence or absence of NA in the medium. From these experiments is concluded that 5-methoxyindoles are active in the pineal gland itself, but that a very complex mechanism of regulation exists, involving the sympathetic innervation and the 5-methoxyindoles. For details see Haldar-Misra and Pévet (1983a, 1983b).

The influence of testosterone on the peptide/protein secretion in in vitro was also studied. Testosterone only induces a significant increase in GV when the pineals of mice and rats are cultured in a NA-free medium. For details see Haldar-Misra and Pévet (1983c).

The influence of luteinizing-hormone-releasing-hormone (LHRH) was studied, using the same in vitro culture system. Here it was also cleary demonstrated that LHRH acts on the synthetic activity of the pineal gland, using the same parameters as described above. For details see Haldar-Misra and Pévet (1983d).

In cultures of mice pinealocytes, prolactin stimulates the protein/peptide secretion by inducing an increase of the formation of GV in the presence of NA. In the rat pinealocytes this hormone could only stimulate the secretion in a NA-free medium. In cultures of rat pinealocytes, prolactin in the presence of NA strongly stimulated the ependymal-like protein/peptide secretory process. For details see Haldar-Misra and Pévet (1983e)

From the above described experiments can be concluded that although the structure of peptides and/or proteins involved in the secretory process of pinealocytes are not yet known, much more is known about the experimental conditions under which different synthetic compounds and hormones can influence this process in in vivo and in in vitro.

In this field of research, the present data indicate that it is now imperative to study specifically, whether hormone receptors play a role in the above described processes. Receptors can be very important and a disfunction of receptor-biosynthesis could perhaps block different other processes. Schotman et al. (1981) using an in vitro incubation-technique, according to Dunlop et al. (1975), have observed that the protein synthesis activity of the rat pineal gland was greatly dependent on the time of dissection,

showing a maximum at midnight and a minimum at 10.00 am., 2 h after the onset of light. These authors concluded that the overall protein synthesis rate in in vitro exhibited a marked circadian rhythmicity comparable to that of serotonin-N-acetyltransferase. This protein synthesis is, according to these authors, under neural and hormonal control. It was found that the action of corticotrophin (ACTH) applied in physiological concentrations, may mediate protein synthesis by a calcium-dependent release of norepinephrine. However, the pineal may not be unique among the neural structures in this respect, as comparable mechanisms exist in many peripheral target organs and in other areas of the brain.
Nevertheless, the observed dramatic effects after hypophysectomy are indicative of an important role for the circulating hypophyseal principles on the protein synthesis of the pineal, as was stated by these authors.

GENERAL DISCUSSION

The original discovery of a vasotocin-like activity in pineal gland extracts by Milcu et al. (1983) has been followed by numerous conflicting reports. Neacşu (1972) asserted, after chemical analysis, that the observed antigonadotropic activity was to be ascribed to a peptide different from AVT and composed of 14 amino acids, termed by this author as E5 (MW≃2000).
Concerning the bovine pineal fraction E5, our present findings do not allow the conclusion that the reported biological activities (Neacşu 1972; Vaughan et al. 1980, 1982) are due to a single pure peptidic antigonadotropic hormone, as the E5-preparation studied by us contains large amounts of proteic material (MW 100 to 10 K). See for details Noteborn et al. (1982) and Ebels (1983).
Our results, obtained in the last years, furthermore demonstrated that (after purification and characterization by HPLC) the bovine pineal fraction E5 also contains immunoreactive AVP and OT. No evidence could be obtained for the presence of AVT nor for the presence of another peptide ending in Pro-Arg-Gly(NH_2).
From experiments with antibodies against different peptides, Pévet (1983) concluded that these antibodies are able to react in some pineal cells with an unidentified compound which he assumes is synthesized in the pineal gland. However, Rix et al. (1981) postulated that the immunocytochemical reaction with different antibodies against comparable peptides is found in the extracellular perivascular space of the pineal and not in a distinct cell population. If that is true, the possibility of a pseudopositive immunostaining reaction may be present in the pineal. In our opinion these contradictory findings call for a critical reevaluation of the hypothesis that the immunological results in the mammalian pineal gland indicate the presence of a large common precursor molecule that is cleaved by enzymes in order to produce a number of smaller active compounds with a different biological activity (Pévet, 1982).
In the last years, several publications have appeared in which the pineal mediated effects of short photoperiods on the secretion of prolactin and gonadotropins of seasonal breeders such as hamsters were studied. For a survey of the literature see Reiter (1980), Benson and Ebels (1981).
Steger et al. (1983) concluded from the results of their experiments with male golden Syrian hamsters, that short photoperiod exposure does not reduce the pituitary-ability to secrete LH or FSH, but that the secretion of Prl is decreased during a restricted period. These authors think that the endocrine changes induced by short photoperiod exposure, are due to functional alterations at multiple sites within the hypothalamic-pituitary-gonadal system. From a very recent publication of Steger et al. (1984) it

it is now known that there are different interactions of pineal substances with the neuroendocrine axis of male Syrian Hamsters. These authors conclude from these experiments that short photoperiod-induced gonadal atrophy in the Syrian hamster is associated with pineal-dependent and pineal-independent changes in hypothalamic neurotransmitter turnover and hypothalamic LHRH-content.
It is possible that one of these pineal compounds which acts on the hypothalamus is a peptide or contains a peptide-moiety indispensible for the biological activity (Matthews and Benson, 1983 and Benson et al. (1983). However, one has to keep in mind that when working with rather crude pineal extracts, they contain a number of hormones which could cause the observed biological activities. Therefore, extracts of control tissues have to be used in order to confirm whether the effects of pineal extracts are caused by pineal"specific" compound(s) or e.g. by hypophyseal principles.
The purification process of a pineal compound, which is trypsin- and chymotrypsin-sensitive which causes and increase in the hypothalamic dopamine de novo synthesis is in an advanced stage. The ultimate purification and characterization of this compound will depend on several circustances, amongst other, the amount of compound present in one pineal. As far as can be judged at this moment, it is thought that several kilograms of pineals are needed. The structure elucidation of this peptidic pineal compound could be of great significance for future pineal research in relation to the hypothalamic-hypophyseal-gonadal axis.
From the results of experiments carried out at the ultrastructural level, it can be concluded that a feed-back mechanism may exist between the peptidic/proteic processes of the pineal and different hypophyseal hormones.
In our opinion, future research should mainly be directed towards two goals:

1. The isolation and identification of (a) pineal peptidic compound(s) which probably influences, by (its) their effect on the syntnesis of hypothalamic catecholamines, plasma Prl- and LH-levels.
2. The elucidation of peptidic/proteic compounds involved in a possible reciprocal relationship between the adenohypophysis and the pineal gland.

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REFERENCES

- Ariëns Kappers, J. (1979) Short history of pineal discovery and research. In: The Pineal of Vertebrates Including Man. Ariëns Kappers, J., Pévet, P. eds. Progr. Brain Res. Vol. 52, Elsevier/North-Holland, Biomedical Press, Amsterdam, pp. 3-22.
- Benson, B. (1977) Current status of pineal peptides. Neuroendocrinology 24, 241-258.
- Benson, B., Ebels, I. (1981) Other pineal peptides and related substances - physiological implications for reproductive biology. In: The Pineal Gland, Vol. II Reproductive Effects. Reiter, R.J. ed. CRC Press Inc., Boca Raton (Fla) U.S.A. pp. 165-187.
- Benson, B., Larsen, B.R., Findell, P.R., Orstead, K.M. (1983) Participation of pineal peptides in reproduction. In: Role of Peptides and Proteins in Control of Reproduction. McCann, S.M., Dhindsa, D. eds. Elsevier Science Publishing Co. Inc., New York, pp. 111-130.
- Czernichow, P., Reinharz, A., Vallotton, M.B. (1974) Immunochemical analysis of rabbit antibodies against vasopressin. Immunochemistry 11, 47-43.
- Collin, J.-P. (1979) Recent advances in pineal cytochemistry. Evidence of the production of indoleamines and proteinaceous substances by photoreceptor cells and pinealocytes in Amniota. In: The Pineal of Vertebrates Including Man, Ariëns Kappers, J., Pévet, P. eds. Progr. Brain Res. Vol. 52, Elsevier/North-Holland, Biomedical Press, Amsterdam, pp. 271-296.
- Collin, J.-P. (1981) New data and vistas on the mechanism of secretion of proteins and indoles in the mammalian pinealocyte and its phylogenetic precursors; the pinealin hypothesis and preliminary comments on membrane traffic. In: The Pineal Organ. Oksche, A., Pévet, P. eds. Elsevier/North-Holland, Amsterdam-New York-Oxford, pp. 187-210.
- Collin, J.-P., Oksche, A. (1981) Structural and functional relationships in the non-mammalian pineal organ. In: The Pineal Gland, Vol. I Anatomy and Biochemistry. CRC Press Inc., Boca Raton (Fla), U.S.A., pp. 27-68.
- Dogterom, J., Wimersma Greidanus, T.B. van, Swaab, D.F. (1977) Evidence for the release of vasopressin and oxytocin into the cerebrospinal fluid: measurements in plasma and CSF of intact and hypophysectomized rats. Neuroendocrinol. 24, 108-118.
- Dubé, D., Kelly, P.A., Pelletier, G. (1980) Comparative localization of prolactin binding sites in different rat tissues by immunohistochemistry, radio-autography and radioreceptor assay. Mol. Cell. Endocrinol. 18, 109-122.
- Dunlop, D.S., Elden W. van, Lajtha, A. (1975) Optimal conditions for protein synthesis in incubated slices of rat brain. Brain Res. 99, 303-318.
- Ebels, I. (1983) Peptidic and proteic substances isolated from pineals and their relation to the hypophyseal-hypothalamic-gonadal axis. In: The Pineal Gland and its Endocrine Role. Axelrod, J., Fraschini, F., Velo, G.P. eds. Plenum Press, New York, London, pp. 178-198.
- Ebels, I., Benson, B. (1978) A survey of the evidence that unidentified pineal substances affect the reproductive system in mammals. In: The Pineal and Reproduction. Reiter, R.J. ed. Progr. in Reprod. Biol. Vol. 4, S. Karger, Basel, pp. 51-89.
- Ellis, S. (1961) Studies on the serial extraction of pituitary proteins. Endocrinology 69, 554-560.
- Haldar-Misra, C., Pévet, P. (1982) The influence of noradrenaline on the process of protein/peptide secretion in the mammalian pineal organ. Cell Tissue Res. 224, 33-44.
- Haldar-Misra, C., Pévet, P. (1983a) Influence of melatonin on the process

of protein and/or peptide secretion in the pineal gland of the rat and hamster. Cell Tissue Res. 231, 73-82.
- Haldar-Misra, C., Pévet, P. (1983b) The influence of different 5-methoxyindoles on the process of protein/peptide secretion characterized by the formation of granular vesicles in the mouse pineal gland. Cell Tissue Res. 230, 113-126.
- Haldar-Misra, C., Pévet, P. (1983c) Influence of testosterone on protein/peptide secretion as characterized by the formation of granular vesicles in rat and mouse pinealocytes. An in vitro study. J. Neural Transm. 57, 111-120.
- Haldar-Misra, C., Pévet, P. (1983d) The influence of luteinizing hormone-releasing hormone (LHRH) on the process of protein and/or peptide secretion characterized by the formation of granular vesicles in mammalian pinealocytes. Cell Tissue Res. 232, 529-538.
- Haldar-Misra, C., Pévet, P. (1983e) Influence of prolactin on the process of protein and/or peptide secretion in mouse and rat pinealocytes. An in vitro study.J. Neural. Transm. 58, 245-259.
- Hwang, P., Guyda, H., Friezen, H. (1972) Purification of human prolactin. J. Biol. Chem. 247, 1955-1958.
- Jiang, N.S., Wilhelmi, A.E. (1965) Preparation of bovine, ovine and porcine prolactin. Endocrinology 77, 150-154.
- Karasek, M. (1974) Ultrastructure of rat pineal gland in organ culture; influence of norepinephrine, dibutyryl cyclic adenosine 3',5'-monophosphate and adenohypophysis. Endokrinologie 64, 106-114.
- Karasek, M. (1978) Pineal-adenohypophysial relationships in the rats. An ultrastructural contribution. Endokrynologia Polska 29, 371-379.
- Karasek, M., Marek, K. (1978) Influence of gonadotropic hormones on the ultrastructure of rat pinealocytes. Cell Tissue Res. 188, 133-141.
- Karasek, M., Pawlikowski, M., Ariëns Kappers, J., Stepien, H. (1976) Influence of castration followed by administration of LHRH on the ultrastructure of rat pinealocytes. Cell Tissue Res. 167, 325-339.
- Karasek, M., King, T.S., Petterborg, L.H., Hansen, J.T., Bartke, A., Reiter, R.J. (1982a) Dense-core vesicles in the mammalian pinealocyte and their relation to secretory processes. Texas Soc. for Electron Microscopy 13, 13-19.
- Karasek, M., Lewinski, A., Hansen, J.T., Reiter, R.J. (1982b) Influence of hypophysectomy and prolactin on the rat pinealocyte: a quantitative ultrastructural study. Reprod. Nutr. Dévelop. 22, 785-792.
- Matthews, M.J., Benson, B. (1983) Inactivation of pineal antigonadotropin by proteolytic enzymes. J. Endocrinol. 56, 339-340.
- Milcu, S.M., Pavel, S., Neacşu, C. (1963) Biological and chromatographic characterization of a polypeptide with pressor and oxytocic activities isolated from bovine pineal gland. Endocrinology 72, 563-566.
- Neacşu, C. (1972) The mechanism of antigonadotropic action of a polypeptide extracted from a bovine pineal gland. Rev. Roum. Physiol. 9, 161-169.
- Noteborn, H.P.J.M., Ebels, I., Pévet, P., Reinharz, A.C., Neacşu, C., Salemink, C.A. (1982) Comparison of some peptidic and proteic ovine pineal fractions with a bovine pineal E5 fraction. J. Neural Transm. 55, 27-44.
- Pévet, P. (1979) Secretory processes in the mammalian pinealocytes under natural and experimental conditions. In: The Pineal of Vertebrates Including Man. Ariëns Kappers, J., Pévet, P. eds. Progr. Brain Res. Vol. 52, Elsevier/North-Holland, Biomedical Press, Amsterdam, pp. 149-194.
- Pévet, P. (1981a) Ultrastructure of the mammalian pinealocyte. In: The Pineal Gland. Vol I Anatomy and Biochemistry, Reiter, R.J. ed. Boca Raton (Fla) U.S.A., CRC Press, pp. 121-154.

- Pévet, P. (1981b) Peptides in the pineal gland of vertebrates: ultrastructural histochemical and radioimmunological aspects. In: The Pineal Organ. Oksche, A., Pévet, P. eds. Elsevier/North-Holland, Biomedical Press, Amsterdam-New York-Oxford, pp. 211-235.
- Pévet, P. (1982) Pineal peptides in the fetus and in young mammals. In: Melatonin Rhythm Generating Systems. Klein, D.C. ed. S. Karger, Basel, pp. 155-181.
- Pévet, P. (1983) The different classes of proteic and peptidic substances present in the pineal gland. In: The Pineal Gland and its Endocrine Role. Axelrod, J., Fraschini, F., Velo, G.P. eds. Plenum Press, London, pp.113-149.
- Reinharz, A.C., Vallotton, M.B. (1977) Presence of two neurophysins in the human pineal gland. Endocrinology 100, 994-1001.
- Reinharz, A.C., Vallotton, M.B., Pévet, P., Dogterom, J. (1981) Neurophysins and neurohormones in the mammalian pineal gland. In: Pineal Function. Matthews, C.D., Seamark, R.F. eds Elsevier/North-Holland, Biomedical Press, Amsterdam, pp. 113-122.
- Reinharz, A.C., Noteborn, H.P.J.M., Klein, M., Vallotton, M.B., Ebels, I., Pévet, P. (submitted for publication) Presence of immunoreactive neurophysins of a higher molecular weight in addition to the 10.000 form in the ovine pineal gland.
- Reiss, M., Davis, R.H., Sideman, M.B., Mauer, I., Plichta, E.S. (1963) Action of pineal extracts on the gonads and their function. J. Endocrinol. 27, 107-118.
- Reiter, R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. Endocrine Reviews 1, 109-131.
- Rix, E., Hackenthal, E., Hilgenfeldt, H., Taugner, R. (1981) Neuropeptides in the pineal gland; a critical immunocytochemical study. Histochemistry 72, 33-38.
- Schotman, P., Allaart, J. Gispen, W.H. (1981) Pineal protein synthesis highly sensitive to ACTH-like neuropeptides. Brain Res. 219, 121-135.
- Steger, R.W., Bartke, A., Goldman, B.D., Soares, M.J., Talamantes, F. (1983) Effects of short photoperiod on the ability of golden hamster pituitaries to secrete prolactin and gonadotropins in vitro. Biol. Reprod. 29, 872-878.
- Steger, R.W., Reiter, R.J. Siler-Khodr, M. (1984) Interactions of pinealectomy and short-photoperiod exposure on the neuroendocrine axis of the male Syrian hamster. Neuroendocrinology 38, 158-163.
- Vaughan, M.K. (1981) Arginine vasotocin and vertebrate reproduction. In: The Pineal Gland. Vol II Reproductive Effects. Reiter, R.J. ed. CRC Press, Boca Raton (Fla) U.S.A., pp. 125-163.
- Vaughan, M.K., Johnson, L.Y., Pévet, P., Neacşu, C., Reiter, R.J. (1980) Effects of a polypeptide (E5) extracted from bovine pineal glands on plasma and pituitary levels of luteinizing hormone (LH) and prolactin in normal and castrated adult male rats. 10th Ann. Meeting Soc. Neurosci. 9-13 november, Cincinnati, U.S.A., Soc. Neurosci. 16, 457.
- Vaughan, M.K., Richardson, B.A., Johnson, L.Y., Reiter, R.J., Pévet, P., Neacşu, C. (1982) Effects of a bovine pineal peptidic fraction E5 on plasma and pituitary levels of LH, FSH and prolactin. Experientia 38, 871-873.

The Pineal Gland
Current State of Pineal Research
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NEURAL CONTROL OF PINEAL INDOLE METABOLISM

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Since Kappers (1960) described the connection between the superior cervical ganglia and the pineal and Moore et al. (1967) found the pathway leading from the retina to the pineal, the innervation of the pineal is intensively studied. This is also due to the fact that the pineal is involved in endocrinological processes which are regulated or are influenced by different environmental conditions. It has been demonstrated that the innervation is mainly adrenergic (Pellegrino de Iraldi and Zieher, 1966; Zieher and Pellegrino de Iraldi, 1966) which means that noradrenaline may be the regulatory product in the pineal. The evidence that noradrenaline and serotonin stimulate adenyl cyclase activity (the synthesizing enzyme for cAMP, the product which mediates hormonal responses) in several tissues lead Weiss and Costa (1967, 1968) to investigate both products on adenyl cyclase activity in the pineal. These authors could demonstrate that noradrenaline stimulates the formation of cAMP most intensive among the different catecholamines tested; whereas the indoleamine serotonin did not influence this synthesis at all. Chronic denervation by superior cervical ganglionectomy did not influence adenyl cyclase activity. However, noradrenaline added to pineals which have been chronically denervated did show a much greater formation of cAMP as compared to pineals with intact innervation (Weiss, 1969; Weiss and Costa, 1967). The effect of noradrenaline on adenyl cyclase activity has been proved to work via β-receptors, because β-blocking agents inhibit the induced enzyme activation (Weiss and Costa, 1968).(Fig. 1)

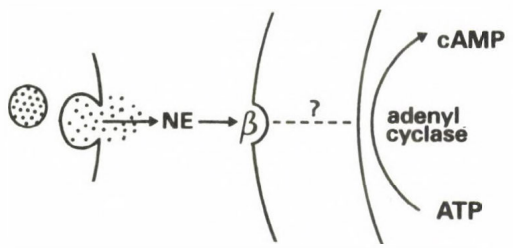

Fig. 1.
Noradrenaline (NE) released from the nerve ending, activates a β-receptor which stimulates adenyl cyclase activity.

The correlation between noradrenaline and cAMP with indole metabolism has been put forward in 1969. In that year Axelrod et al. found a stimulation of melatonin synthesis from tryptophan by noradrenaline. Shein and Wurtman (1969) and Klein et al. (1970) demonstrated that a synthetic analog of cAMP, dibutyryl cAMP (DAMP) mimics the effect of noradrenaline (Fig. 2).

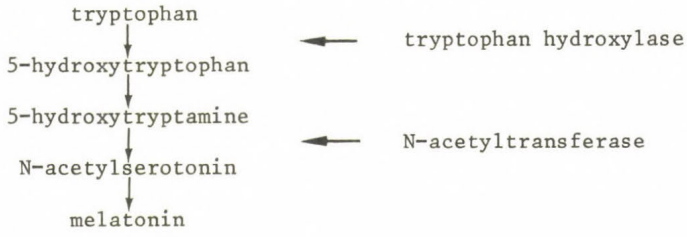

Fig. 2. The synthesis of melatonin from tryptophan.

The most important finding in the regulation of melatonin synthesis is the stimulatory effect of noradrenaline and of DAMP on N-acetyltransferase activity (Klein et al., 1970; Klein and Weller, 1973). Herewith indicating that N-acetylation is the rate limiting step in melatonin synthesis. On the other hand noradrenaline as well as DAMP stimulate serotonin synthesis (Wurtman et al., 1971; Shein and Wurtman, 1969). This stimulation takes place in the hydroxylation of tryptophan (Shein and Wurtman, 1971).

With these findings the importance of noradrenaline - cAMP involvement in indole metabolism is clear. However, one main problem remains by reading this literature: "Why is dibutyryl cAMP used instead of the naturally occuring cAMP? A few investigations have been performed with "natural" cAMP. However, the results are contradictory. In experiments of Klein et al. (1970) no influence on melatonin synthesis could be found. In contrast to these findings Wurtman et al. (1971) described a significant inhibition of the synthesis of melatonin by natural cAMP. The synthetictanalog DAMP, however, caused in these experiments a great stimulation. The explanation of Klein et al. (1970) that the stimulatory effect of DAMP is a result of slower degradation of this product by phosphodiesterase and a more rapid entry in the cell by its dibutyryl tails, is not applicable for the inhibitory activity found by Wurtman et al. (1971).(Fig. 3).

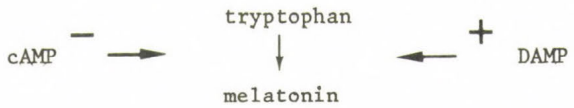

Fig. 3. The discrepancy between the effect of cAMP and DAMP on melatonin synthesis.

The discrepancy between the effect of "natural" cAMP and DAMP lead to experiments performed with "natural cAMP".

The experiments have been performed with male Wistar rats of 140 ± 5g. They were kept under a 12L:12D schedule in the month of July. Every 2 or 3 hours three animals were decapitated and their pineals investigated. The synthesis of N-acetylserotonin and melatonin (Fig. 2) under influence of different concentrations of "natural" cAMP have been determined by the method of Balemans et al. (1981). In this method no substrate is added; acetyl-coenzyme A has been used as acetyl donor.

N-acetylserotonin synthesis

N-acetylserotonin synthesis is completely inhibited when 10^{-5}M cAMP is added to the incubation medium (Fig. 4), whereas a concentration of 10^{-2}M causes a clear stimulation (Fig. 5).

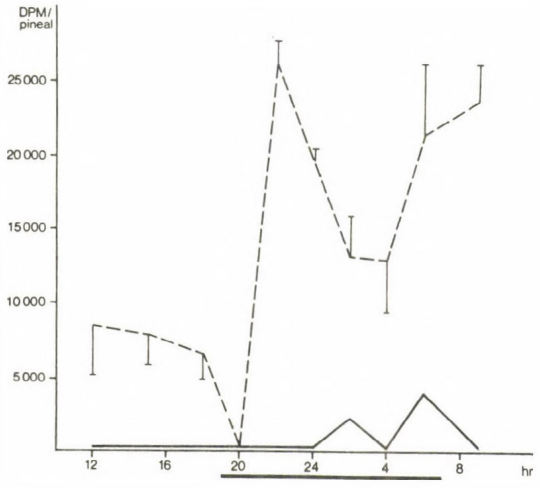

Fig. 4. The influence of 10^{-5}M cAMP on N-acetylserotonin synthesis in the pineal of the young male rat. --- control; —— cAMP treated pineals.

After incubation with 3.10^{-3}M cAMP Wurtman et al. (1971) found a significant inhibition of serotonin synthesis from tryptophan. Using about the same concentration (2.10^{-3}M) of cAMP in experiments performed in the month of August, also a complete inhibition of N-acetylserotonin synthesis could be observed (Fig. 6). The question arises now:

1. which effects have to be considered as physiological; those performed with "natural" cAMP or those obtained with DAMP.
2. If "natural" cAMP is concerned, than the possibility of a dual effect must not be excluded. That means: inhibition with low concentrations of cAMP and stimulation with high concentrations.
3. On the other hand the effects of low concentrations may be considered as physiological and the effects of high concentrations probably as

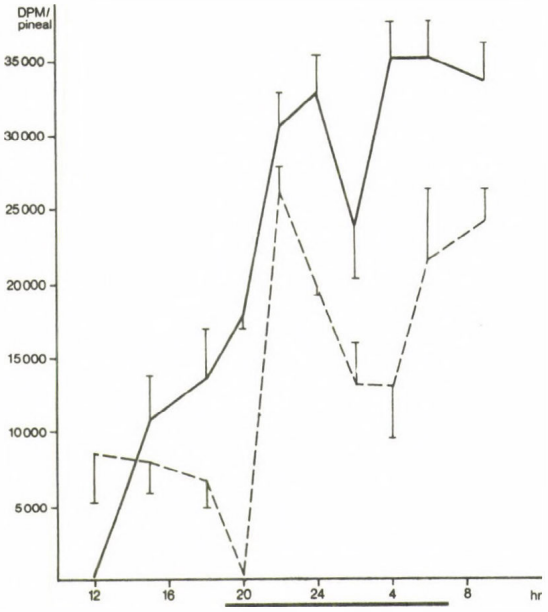

Fig. 5. The influence of 10^{-2}M cAMP on N-acetylserotonin synthesis in the pineal of the young male rat. --- control; —— cAMP treated pineals.

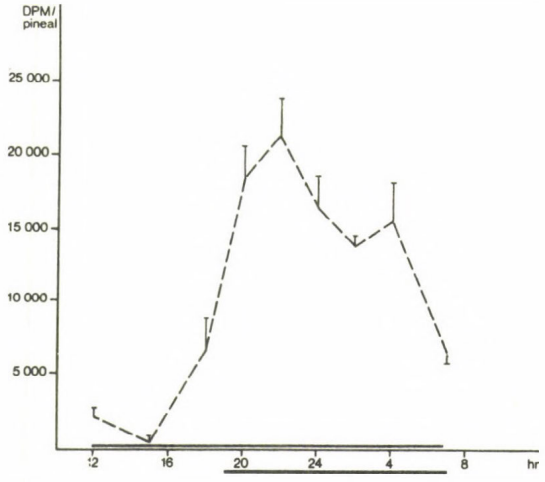

Fig. 6. The influence of 2.10^{-3}M cAMP on N-acetylserotonin synthesis in the pineal of the young male rat. --- control; —— cAMP treated pineals.

pharmacological processes. The statement that "natural" cAMP hardly or not penetrates the cell membrane is disputable since Berg and Klein (1971) found that 30% of natural cAMP penetrates the cell after a 4 hour period. These authors, however do not mention investigations performed between 0 and 4 hours. The lipophylic character of the synthetic analog DAMP may cause an easier entry in the cell. Therefore the results of low doses of DAMP probably may be comparable with the results of high doses of cAMP. The question, however, remains if this is a physiological process or not, since low doses produce an inhibitory effect. The latter probably can be explained by the decreased serotonin (the precursor of N-acetylserotonin) synthesis by cAMP as has been observed by Wurtman et al. (1971).

Melatonin synthesis

Melatonin synthesis under influence of 10^{-5} (Fig. 7) and 10^{-2}M cAMP (Fig. 8) shows about the same pattern over a 24 hour period. A stimulation of synthesis at 04 and 06h and an inhibition or no effect during the rest of the day/night period. The mean synthesis over a 24 hour period seems to be higher if low concentration is used. This result probably may indicate that high concentrations can inhibit melatonin synthesis. Therefore also experiments have been performed during the night period with an extremely high concentration (10^{-1} M). In these experiments melatonin synthesis is determined in the pineal tissue (Fig. 9) and in the incubation medium (Fig. 10) separately. It is clear that "natural" cAMP inhibits melatonin synthesis completely.(experiments have been performed in the month of May)

The results presented can be compared with those obtained from experiments in which melatonin synthesis is determined after being influenced by cAMP. That means no effect (Klein et al., 1970) or an inhibition of melatonin synthesis (Wurtman et al.,1971). For melatonin synthesis we can not find a comparison in effect between cAMP and the synthetic analog DAMP. The effect at 04 and at 06h by using a low concentration of cAMP may suggest a stimulation, an effect which is generally described for DAMP. Experiments, however, with lower concentrations of cAMP have not been performed.

The discrepancies between cAMP and DAMP on N-acetylserotonin and melatonin synthesis need to look further to the influence of noradrenaline. In literature also contradictory results are described concerning the influence of noradrenaline on indole metabolism. Axelrod et al. (1969) and Klein et al. (1970) found a stimulation of melatonin synthesis from tryptophan; and Berg and Klein (1971) described a stimulation of N-acetyltransferase activity by using serotonin as a substrate. In contrast to the above mentioned results Weiss (1968) demonstrated that noradrenaline inhibits hydroxyindole-O-methyltransferase (HIOMT) activity (the melatonin synthesizing enzyme). This observation is in agreement with the data of Brownstein and Heller (1968) who found a decrease in HIOMT activity after nerve stimulation. Also Heydorn et al. (1983) described paradoxically to the results on N-acetyltransferase (Deguchi, 1973) a reduction of catecholamine induced melatonin production after continuous light.

Therefore experiments identical to those performed with cAMP have been done with noradrenaline. Young male Wistar rats of 140-5g have been used. They were kept under a 12L:12D schedule. The synthesis of N-acetylserotonin and of melatonin under influence of different concentrations of noradrenaline and in different months of the year has been determined over

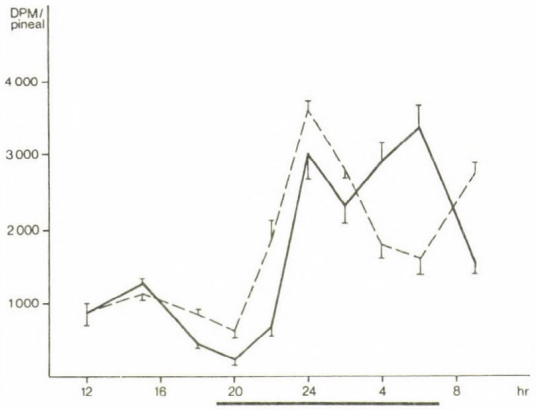

Fig. 7. The influence of 10^{-5}M cAMP on the synthesis of melatonin in the pineal of the young male rat. --- control; —— cAMP treated pineals.

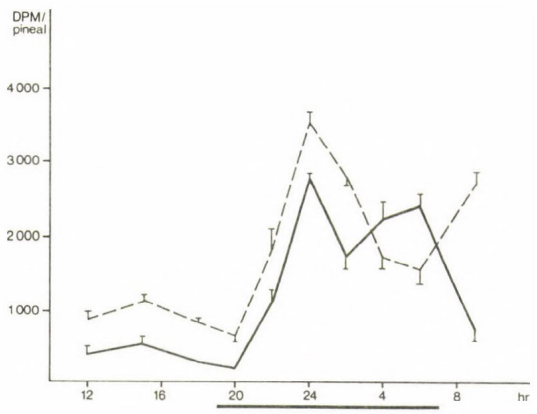

Fig. 8. The influence of 10^{-2}M cAMP on the synthesis of melatonin in the pineal of the young male rat. --- control; —— cAMP treated pineals.

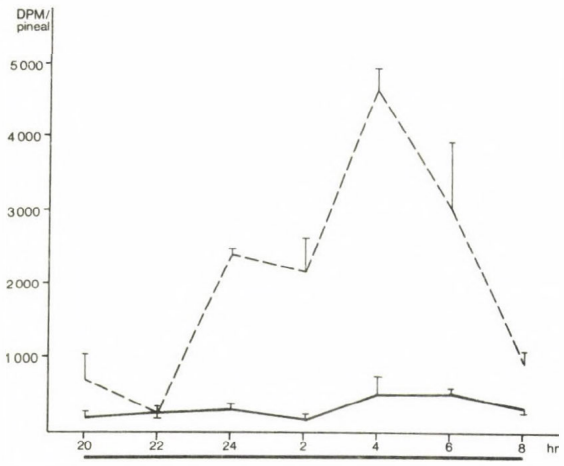

Fig. 9. The influence of 10^{-1}M cAMP on the synthesis of melatonin in the pineal of the young male rat. Melatonin present in pineal tissue.
--- control; —— cAMP treated pineals.

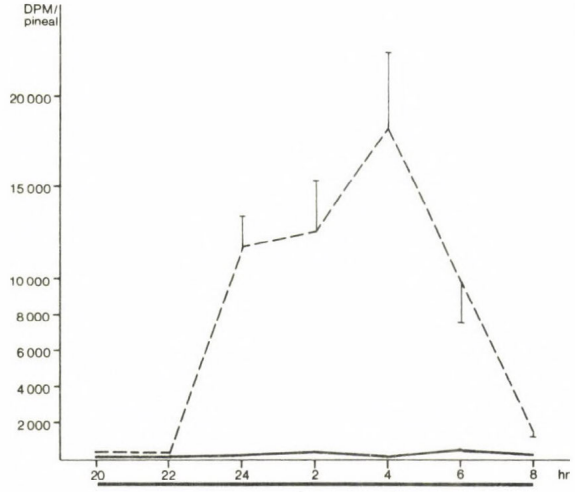

Fig. 10. The influence of 10^{-1}M cAMP on the synthesis of melatonin in the pineal of the young male rat. Melatonin present in the incubation medium.
--- control; ——cAMP treated pineals.

a 24h period with the method described by Balemans et al. (1981). No substrate is added; acetyl-coenzyme A is used as acetyl donor. For each experiment the mean value of synthesis over a 24h period (N=39; three animals have been investigated every two hours) is presented in the tables.

N-acetylserotonin synthesis

Under influence of 10^{-5}M noradrenaline N-acetylserotonin synthesis is stimulated in the month of June (16,75%). If a higher concentration is used (10^{-2}M) than an inhibition can be observed (Table 1.).

Table 1. The mean synthesis of N-acetylserotonin over a 24h period.

noradrenaline concentration	control pineals	N	noradrenaline treated pineals	N
10^{-5}M	10166 dpm (100%)	39	11869 dpm (116,75%)	39
10^{-2}M	10166 dpm (100%)	39	8045 dpm (79.14%)	39

Melatonin synthesis

Melatonin synthesis is stimulated by 10^{-5}M noradrenaline, whereas 10^{-2}M causes no effect (Table 2.)

Table 2. The mean synthesis of melatonin over a 24h period.

noradrenaline concentration	control pineals	N	Noradrenaline treated pineals	N
10^{-5}M	2397 dpm (100%)	39	2990 dpm (124.75%)	39
10^{-2}M	2397 dpm (100%)	39	2468 dpm (102.96%)	39

From these results it may be concluded that low concentrations of noradrenaline produce an increase in N-acetylserotonin as well as melatonin synthesis. This result is in agreement with the general findings with low concentrations of noradrenaline. The stimulation of N-acetylserotonin synthesis does fit in the stimulatory effect of DAMP, which is generally described, however, it does not in the inhibitory effect as has been found for 10^{-5}M "natural" cAMP.

This contradiction probably must be described to the different products used, cAMP or DAMP. The poesebility that contradictory results are caused by using animals of different ages must not be excluded. On the other hand it can be observed from Fig. 7 and 8 and Fig. 9 and 10 that melatonin synthesis differs enormously between May and July. So that the time of the year in which the experiments have been performed also is important.

Therefore preliminary experiments with noradrenaline have been performed in the months June, July and August with male Wistar rats of 140±5g They were kept under 12L:12D conditions. The synthesis of N-acetylserotonin and of melatonin under influence of 2.10^{-3}M noradrenaline has been determined with the method described by Balemans et al. (1981).No substrate has been added; acetyl-coenzyme A has been used as acetyl donor.

N-acetylserotonin and melatonin synthesis

N-acetylserotonin as well as melatonin synthesis show, without being influenced by noradrenaline, an accelerated decrease in July and August.

Table 3. The mean synthesis of N-acetylserotonin and of melatonin.

	N	June	N	July	N	August
N-acetylserotonin	39	14105dpm (100%)	39	10166 dpm (72.07%)	39	5505 dpm (39.03%)
melatonin	39	2397dpm (100%)	39	1759 dpm (73.38%)	39	1108 dpm (46.22%)

If noradrenaline is added to the incubation medium also a reduction of N-acetylserotonin synthesis can be found (Table 4.). Melatonin synthesis is slightly stimulated in June, but is reduced in July and August. It can be observed that the decrease in N-acetylserotonin and melatonin synthesis in July and August is much stronger when noradrenaline is added to the incubation medium.

More or less identical results have been described for N-acetyltransferase activity influenced by 5-hydroxytryptophan. 5-Hydroxytryptophan causes an accelerated fall of N-acetyltransferase activity induced by 1-propranolol (a ß-adrenergic blocking agent); but also the stimulation of this enzyme by noradrenaline is reduced by 5-hydroxytryptophan. In contrast to these findings Namboodiri et al. (1983) found an elevated melatonin synthesis after 5-hydroxytryptophan treatment. Besides 5-hydroxytryptophan also serotonin can influence indole metabolism . Weiss (1968) described an inhibitory effect of serotonin on HIOMT activity and Weiss and Costa (1968) mentioned an inhibition of serotonin on noradrenaline induced stimulation of adenyl cyclase activity. These investigations indicate that the substrate added has more capabilities than being substrate alone.

Table 4. The mean synthesis of N-acetylserotonin and melatonin of one day/night in the months of June, July and August.

	control pineals	noradrenaline treated pineals		
	June	June	July	August
N-acetylserotonin	14105 dpm (100%)	11707 dpm (83%)	6912 dpm (49%)	3247 dpm (23%)
melatonin	2397 dpm (100%)	2588 dpm (108%)	1090 dpm (45.5%)	542 dpm (22.6%)

Discrepancies can also be observed in experiments performed with GABA. This neurotransmitter like substance is present in the pineal (Schon et al. 1975; Labella et al., 1968; Waniewski and Suria, 1977) and has been tested on indole metabolism by several authors. Mata et al. (1976) and Wheler and Klein (1980) could not find an effect on N-acetyltransferase activity in the rat pineal. However, in the rat a stimulatory activity of GaBA on N-acetylserotonin and melatonin synthesis has been described by Balemans et al. (1983). Whereas in the bovine pineal GABA caused an inhibition on N-acetyltransferase activity (Ebadi and Chan, 1980; Chan and Ebadi, 1980).

concluding remarks.

The contradictory results in the regulation of indole metabolism caused by cAMP, noradrenaline and GABA applicated in different concentrations and during different months of the year strongly suggest a cooperation between these compounds in which the ratio may cause the effect: stimulatory or inhibitory.

From literature and from present results it is clear that N-acetylserotonin and melatonin are not always synthesized parallel to each other. This might be an indication of a separate regulation or/and the indication of another pathway leading to melatonin (f.e. via 5-methoxytryptamine)

Recent findings, such as the implication of α-adrenergic receptors, which potentiate the β-adrenergic stimulation (Klein et al.,1983); the existance of a N-acetyltransferase inactivating substance (Chan and Ebadi 1981); the β-adrenergig control of HIOMT (Sugden and Klein, 1983); and the involvement of prostaglandins in the regulation of indole metabolism (Cardinali et al., 1982) makes indole metabolism much more complicated than it already was.

References

Axelrod J., Shein H.M., Wurtman R.J.: Stimulation of melatonin synthesis from tryptophan by noradrenaline in rat pineal in organ culture. Proc. Natl. Acad. Sci. 62, 544- 549 1969.

Balemans M.G.M., Smith I., de Reuver G.F.: Changes in the circadian rhythmicity of N- and O-acetyltransferase activities in the pineal gland of 38 day old male Wistar rats when examined under white, red and green light. J.Neur. Transm. 52, 83- 93 1981.

Balemans M.G.M., Mans D., Smith I, van Benthem J.: The influence of GABA on the synthesis of N-acetylserotonin, melatonin, O-acetyl-5-hydroxytryptophol and O-acetyl-5-methoxytryptophol in the pineal gland of the male Wistar rat. Reprod. Nutr. Dévelop., 23, 151- 160 1983

Berg G.R., Klein D.C.: Pineal gland in organ culture. Role of adenosine 3,5 monophosphate in the regulation of radiolabeled melatonin production. Endocrinology, 89, 453- 464 1971.

Brownstein M.J., Heller A.: Hydroxyindole-O-methyltransferase activity: Effect of sympathetic nerve stimulation. Science 162, 367- 368 1968.

Cardinali D.P., Ritta M.N., Pereyra E., Solveyra C.G.: Role of prostaglandins in rat pineal neuroeffector junction. Changes in melatonin and norepinephrine release in vitro. Endocrinology, 111, 530- 534 1982.

Chan A., Ebadi M.: The kinetics of norepinephrine-induced stimulation of serotonin N-acetyltransferase in bovine pineal gland. Neuroendocrinology, 31, 244- 251 1980.

Chan A., Ebadi M.: Reciprocal relationship between the concentrations of serotonin and the activity of serotonin N-acetyltransferase in rat pineal gland in culture. Endocr. Res. Comm. 8, 25-44 1981.

Chan A., Ebadi M.: Evidence for existence of a serotonin N-acetyltransferase inactivating substance in rat pineal gland. Endocr. Res. Comm. 8, 205- 227 1981.

Deguchi T.: Control of the beta-adrenergic receptor in the elevation of adenosine cyclic 3,5-monophosphate and induction of serotonin N-acetyltransferase in rat pineal glands. Molec. Pharmacol., 9, 184- 190 1973.

Ebadi M., Chan A.: Characteristics of GABA binding sites in bovine pineal gland. Brain Res. Bull., 5, suppl. 2, 179- 187. 1980.

Heydorn W., Lucki I., Frazer A.: Continuous light paradoxically reduces catecholamine-induced melatonin production. Brain Research, 267, 175- 178 1983.

Kappers J.A.: The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. Z.Zellforsch., 52, 163- 215 1960.

Klein D.C., Berg G.R., Weller J., Glinsmann W.:Pineal gland:Dibutyryl Cyclic Adenosine Monophosphate stimulation of labeled melatonin production Science, 167, 1738- 1740 1970.

Klein D.C., Berg G.R., Weller J.; Melatonin synthesis: Adenosine 3,5-monophosphate and norepinephrine stimulate N-acetyltransferase. Science, 168, 979- 980 1970.

Klein D.C., Weller J.L.: Adrenergic adenosine 3,5-monophosphate regulation of serotonin N-acetyltransferase activity and the temporal relationship of serotonin N- acetyltransferase activity to synthesize N-acetylserotonin and melatonin in the cultured rat pineal gland. J. Pharmac. Exp. Therap. 186, 516-527 1973.

Klein D.C., Sugden D., Weller J.L.: Postsynaptic alpha adrenergic receptors potentiate the beta-adrenergic stimulation of pineal serotonin N-acetyltransferase. Proc. Natl. Acad. Sci. 80, 599- 603 1983.

Labella F., Vivian S., Queen G.: Abundance of cystathione in the pineal body. Free amino acids and related compounds of bovine pineal, and anterior pituitary and brain. Biochem. Biophys. Acta, 158, 286- 288 1968

Mata M.M., Schrier B.K., Klein D.C., Weller J.L., Chiou C.T.: On GABA function and physiology in the pineal gland. Brain Research, 188, 383- 394 1976.

Moore R.Y., Heller A., Wurtman R.J., Axelrod J.: Visual pathway mediating pineal response to environmental light. Science, 155, 220- 223 1867.

Namboodiri M.A.A., Sugden D., Klein D.C., Mefford I.N.: 5-Hydroxytryptophan elevates serum melatonin. Science, 221, 659- 661 1983.

Pellegrino de Iraldi A., Zieher L.M.: Noradrenaline and dopamine content of normal, decentralized and denervated pineal gland of the rat. Life Sci., 5, 149 - 154 1966.

Schon F., Beart P.M., Chapma D., Kelly J.S.,: On GABA metabolism in the gliocyte cells of the rat pineal gland. Brain Research, 85, 479- 490 1975.

Shein H.M., Wurtman R.J.:Cyclic adenosine monophosphate: stimulation of melatonin and serotonin synthesis in cultured rat pineals. Science, 166, 519- 520 1969.

Shein H.M., Wurtman R.J.:Stimulation of tryptophan 5-hydroxylation by norepinephrine and dibutyryl adenosine 3,5-monophosphate in rat pineal organ cultures. Life Sci. 10, 935- 940 1971.

Sugden D., Klein D.C.: Beta-adrenergig receptor control of rat pineal hydroxyindole-O-methyltransferase. Endocrinology, 113, 348- 353 1983.

Waniewski R., Suria A.: Alterations in GABA content in the rat superior cervical ganglion and pineal gland. Life Sci. 21, 1129- 1142 1977.

Weiss B.: Discussion of the formation, metabolism and physiologic effects of melatonin. Advanc. Pharmacol. 6A, 152- 155 1968.

Weiss B.: Effects of environmental lighting and chronic denervation on the activation of adenyl cyclase of rat pineal gland by norepinephrine and sodium fluoride. J. Pharmac. Exp. Therap. 168, 146- 152 1969.

Weiss B., Costa E.: Adenyl cyclase activity in rat pineal gland: Effects of chronic denervation and norepinephrine. Science 156, 1750-1752 1967.

Weiss B., Costa E.: selective stimulation of adenyl cyclase of rat pineal gland by pharmacologically active catecholamines. J. Pharmac. Exp. Therap. 161, 310- 319 1968.

Wheler G.H.T., Klein D.C.: Taurine release from the pineal gland stimulated via a beta-adrenergic metabolism. Brain Research, 187, 155- 164 1980

Wurtman R.J., Shein H.M., Larin F.: Mediation by beta-adrenergig receptors of effect of norepinephrine on pineal synthesis of serotonin and melatonin. J. Neurochem. 18, 1683- 1687 1971.

Zieher L.M., Pellegrino de Iraldi A.: Central control of noradrenaline content in rat pineal and submaxillary glands. Life Sci. 5, 155- 161 1966.

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COMPLEX CONTROL OF THE CIRCADIAN RHYTHM IN PINEAL MELATONIN PRODUCTION

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1. REGULATION OF THE PINEAL MELATONIN RHYTHM

1.1 Biochemical control of the pineal melatonin rhythm

Melatonin in the pineal gland is formed first by acetylation of serotonin to N-acetylserotonin by N-acetyltransferase (NAT) (EC 2.3.1.5) (Weissbach et al. 1961) and then by methylation of N-acetylserotonin to melatonin by hydroxyindole-O-methyltransferase (HIOMT)(EC 2.1.1.4) (Axelrod and Weissbach 1960). In all mammals so far studied, pineal melatonin content and NAT activity exhibit marked daily rhythms with high night levels and low daytime values (Klein 1979). In rats and in Djungarian hamsters, the rhythm in melatonin production is apparently driven by the NAT rhythm (Klein and Weller 1970, Wilkinson et al. 1977, Illnerová et al. 1983). Melatonin concentration starts to change dramatically exactly at the time when NAT activity begins to rise or to decline (Illnerová et al. 1983). The evening NAT and melatonin rise is parallel, as well as the morning decline (Fig. 1). On the contrary, HIOMT does not change significantly through the whole night (Quay 1967, Klein and Moore 1979, Illnerová and Vaněček 1983). Obviously, dramatic changes in NAT activity trigger the beginning and the end of the high melatonin production. Hence the circadian NAT rhythm drives the melatonin rhythm and regulates the duration of the period of elevated night melatonin concentrations. However, as melatonin concetration approaches its maximum at a time when NAT activity still continues to rise, it seems that something else besides NAT activity may limit the maximum night melatonin production. HIOMT activity and concentrations of substrates may belong to these limiting factors (Wurtman and Ozaki 1978).

1.2 Circadian control of the NAT and melatonin rhythms

The daily rhythms in NAT activity and melatonin concentration are truly circadian as they persist under constant lighting conditions, in continuous darkness (Klein and Weller 1970, Ralph et al. 1971, Ralph et al. 1975, Perlow et al. 1981). The rat pineal NAT rhythm is abolished either by denervation of the gland through bilateral superior cervical ganglionectomy or by decentralization of the ganglia (Klein et al. 1971). Hence the rhythm is controlled by a pacemaker located outside the gland,

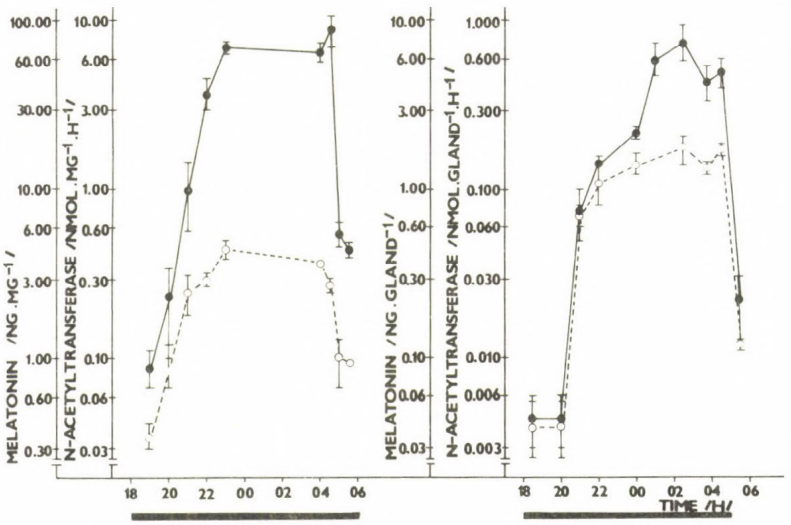

Fig. 1. Circadian rhythm of N-acetyltransferase activity (filled circles) and melatonin concentration (open circles) in the pineal of the rat (left side of the figure) and of the Djungarian hamster (right side). Each point represents mean ±SEM of 4-6 determinations. The black line under abcissa indicates the duration of the dark period. Note that values are expressed on the logarithmic scale.

most probably in the suprachiasmatic nucleus of the hypothalamus as destruction of these nuclei also abolishes the NAT rhythm (Klein and Moore 1979). Signals originating in the hypothalamus are transmitted to the pineal gland by a neural circuit which includes superior cervical ganglia of the sympathetic nervous system. At night in darkness, noradrenaline released from the sympathetic nerve endings in the pineal gland (Brownstein and Axelrod 1974) interacts with the beta-adrenergic receptors on the pineal cell membrane enhancing the production of c AMP which mediates the induction and activation of NAT (Klein and Berg 1970). Beta-adrenergic activation is potentiated by an alpha--adrenergic receptor mechanism (Klein et al. 1983). Activated beta-adrenergic receptors are necessary not only for the evening NAT induction but for the maintenance of the high night NAT activity as well (Deguchi and Axelrod 1972). Due to the circadian control of the NAT rhythm, induction of the high activity in darkness occurs only at night; during day rats are refractory to darkness (Binkley et al. 1974, Illnerová 1974).

2 EFFECT OF LIGHT ON THE NAT AND MELATONIN RHYTHMS

2.1 Suppresive effects of light on the high night melatonin production

Under constant light, the pineal rhythms in NAT activity

and melatonin content are abolished in the rat, chicken and monkey (Klein et al. 1970, Ralph et al. 1975, Perlow et al. 1981). In mammals light may act through the retinohypothalamic projection to the suprachiasmatic nucleus and it may inhibit neuronal transmission necessary to stimulate the pineal gland (Klein and Moore 1979). At night, exposure to sudden light of a sufficiently strong intensity induces a rapid increase in the pineal serotonin concentration and a precipitous decline in the melatonin concentration and NAT activity to daytime levels in all mammals so far studied (Illnerová 1971, Deguchi and Axelbrod 1972, Klein and Weller 1972, Rollay and Niswender 1976, Illnerová et al. 1978, Tamarkin et al. 1979, Vaněček and Illnerová 1982a). Difference in the sensitivity of the pineal gland to light exists among mammals and it may be due to their previous lighting history (Reiter et al. 1983). For example, white light of the intensity of 0.2 lx is sufficiently strong to induce the NAT decline in rats (Vaněček and Illnerová 1982b) while light of the intensity of 2500 lx is necessary to induce the drop in the plasma melatonin concentration in humans (Lewy et al. 1980).

When rats are exposed to light at night, NAT activity declines biphasically: first with a halving time 3.8 min and then with a halving time 70 min (Vaněček and Illnerová 1979). Apparently, the first halving time may correspond to a rapid deactivation of the night enzyme to a less active form after the interruption of beta-adrenoceptors activation and the second halving time may correspond to the cessation of the synthesis of the night enzyme (Vaněček and Illnerová 1980). The melatonin concentration and NAT activity decline rapidly not only when rats are exposed to a prolonged light at night but also when animals are exposed only to a 1-min light pulse (Illnerová et al. 1979). When light of a sufficiently strong intensity is used, even a 0.1-min light pulse may induce the rapid NAT decline in the continuing darkness (Vaněček and Illnerová 1982b). After a 1-min light pulse, NAT activity declines with the same velocity as after beta-blockers administration (Vaněček and Illnerová 1979). When the beta-adrenergic agonist isoproterenol is applied in the course of a 1-min pulse, no NAT decline occurs (Illnerová and Vaněček 1979). Hence, brief light pulses may shut-off or reduce immediately the night noradrenaline release from nerve endings in the rat pineal gland.

The suppressive effect of brief light pulses at night on the high melatonin production cannot be simply explained by a long-term inhibition of the neural transmission to the pineal gland by a prolonged light. Rather, 1-min light pulses might act as phase-resetting signals of the NAT rhythm.

2.2 Entraining effects of 1-min light pulses on the NAT rhythm

Light, besides its suppressive effect on the high night melatonin production, may entrain the endogenous pacemaker controlling the pineal rhythms with environmental lighting. Brief light pulses might act also as entraining signals. When rats maintained under the light-dark regime LD 12:12 are exposed to a 1-min light pulse before midnight, NAT activity, following the initial decline, begins to increase anew to high night values after a lag period as if the evening NAT rise were phase-delayed

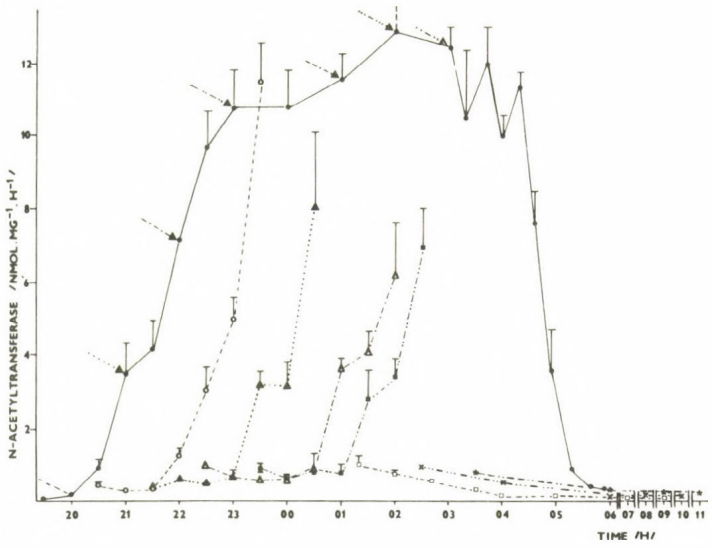

Fig. 2: The N-acetyltransferase rhythm in the course of night when 1-min light pulses were applied. Rats maintained in LD 12: 12, with lights on from 0600 h to 1800 h were either unpulsed (filled circles) or pulsed at 2000 h (open circles), or at 2100 h (filled triangles), or at 2200 h (open triangles), or at 2300 h (filled squares), or at 0100 h (open squares), or at 0200 h (crosses), or at 0300 h (asterisks) and from that time on they were kept in darkness untill killed. Arrows indicate time of the pulses and point to the N-acetyltransferase value in darkness at the moment of the pulse. Data are expressed as means ± S.E.M. of four animals.

(Fig. 2)(Illnerová and Vaněček 1982a,b). After a 1-min light pulse applied past midnight, NAT activity declines rapidly and does not increase to high night values through the rest of night as if the morning NAT decline were phase-advanced. Thus, the boundary between re-inducibility and non-inducibility of NAT by darkness after brief light pulses passes approximately through midnight.

Light pulses applied before midnight might phase-delay the pacemaker driving the NAT rhythm to a phase when NAT activity is still low. Similarly, pulses after midnight might phase--advance the pacemaker to a phase when NAT activity is already low. To prove that 1-min light pulses may serve as effective phase-shifting signals of the pacemaker, we exposed rats to a 1-min light pulse at 0300 h (Fig. 3A) or at 2100 h (Fig. 3B) or we left them unpulsed, thereafter we released them to continuous darkness (DD) to let the rhythms become free-running and we determined the rhythms after one and four days in DD (Illnerová and Vaněček 1982 a,b, Illnerová and Vaněček 1983). Through the whole paper, phase-shifts of the evening NAT rise are ex-

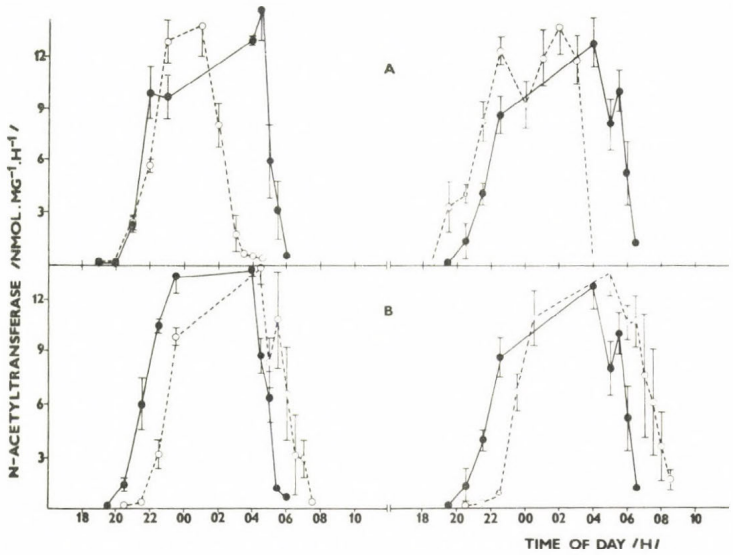

Fig. 3. The N-acetyltransferase rhythm one (left side of the figure) and four days (right side) after application of a 1-min light pulse at 0300 h (upper part of the figure, A) or at 2100 h (lower part, B). Rats maintained in LD 12:12, with lights on from 0600 h to 1800 h, were either exposed to a 1-min light pulse (open circles) or they were left unpulsed (closed circles) and then they were released into constant darkness. Data are expressed as means ± S.E.M. of four animals.

pressed as the time interval between the times when NAT activity reaches the value of 3 nmol mg^{-1} h^{-1} during its rise in pulsed animals and in unpulsed controls. Similarly, phase-shifts of the morning NAT decline are always expressed as the time interval between the times when NAT activity declines to the value of 3 nmol mg^{-1} h^{-1} during its decrease in pulsed animals and in unpulsed controls. One day after the pulse at 0300 h, the evening NAT rise is not phase-shifted and the morning NAT decline is phase-advanced by 2.7 h. Four days after the pulse at 0300 h, the rise is phase-advanced by 1.6 h and the decline by 2.6 h. One day after the pulse at 2100 h, the evening NAT rise is phase-delayed by 1.6 h and the morning NAT decline by 1.3 h. Four days after the pulse at 2100 h, the rise is phase-delayed by 1.7 h and the decline by 1.8 h. In this experimental arrangement, phase-shifts of the NAT rise and decline express phase--shifts of the pacemaker driving the NAT rhythm one and four days after the pulses. As the evening rise and the morning decline do not shift always parallel after the pulses they may possibly be driven by two coupled oscillators: the evening one, controlling the NAT rise and the morning one, controlling the NAT decline.

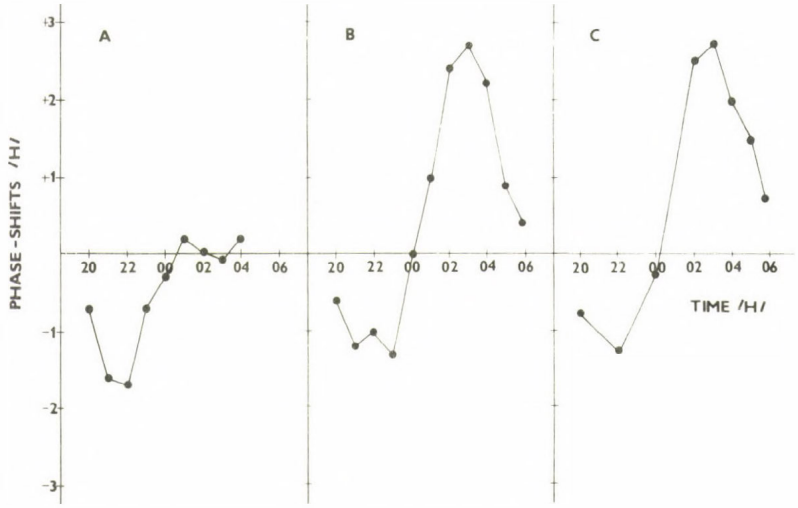

Fig. 4: Phase-responses curves representing phase-shifts of the evening N-acetyltransferase rise (A), of the morning N-acetyltransferase decline (B) and of the boundary between re-inducibility and non-inducibility of N-acetyltransferase (C) one day after presentation of 1-min light pulses. Values of phase-shifts of the evening N-acetlytransferase rise and of the morning N-acetyltransferase decline were taken from Illnerová and Vaněček (1982a), values of phase-shifts of the boundary between N-acetyltransferase re-inducibility and non-inducibility by darkness after light pulses were taken from Illnerová and Vaněček (1984). Abcissa denotes time of pulse presentation. Phase-delays are expressed by the sign -, phase-advances by the sign +.

3. TWO-OSCILLATOR STRUCTURE OF THE PACEMAKER CONTROLLING THE NAT RHYTHM IN THE RAT PINEAL GLAND

3.1 Phase-response curves for shifts of the evening NAT rise and the morning NAT decline one day after application of 1-min light pulses

Phase-response curves (PRCs) indicate how the amount and the sign of a phase-shift, induced by a single stimulus, in our case by a 1-min light pulse, depends on the phase at which the stimulus is applied. If there are two oscillators driving the NAT rhythm, then their PRCs may not be identical. Rats maintained under LD 12:12 were exposed to 1-min light pulses at different night times or they were left unpulsed, then they were released into DD and the next night evening NAT rises and morning NAT declines were determined (Illnerová and Vaněček 1982 a,b). Examples of the phase-shifts are depicted on the left side of

Fig. 3. Phase-shifts of the evening NAT rise and of the morning NAT decline in pulsed animals relative to the pattern of the NAT rhythm in unpulsed controls are summarized in Fig. 4, A, B. The PRC demonstrating phase-shifts of the evening NAT rise one day after the pulses has only phase-delays (Fig. 4, A), while the PRC demonstrating phase-shifts of the morning NAT decline one day after the pulses has phase-delays as well as phase-advances, though phase-advances are more pronounced (Fig. 4, B). From the existence of two different PRCs it is possible to infer that these PRCs represent phase-shifts of two oscillators. Hereafter the evening oscillator will be designated E and the morning oscillator M according to Pittendrigh and Daan (1976) who proposed that E, coupled to dusk, controls the evening component of the locomotor activity in rodents, while M, coupled to dawn, controls the morning component.

3.2 Phase-response curve for shifts of the boundary between re--inducibility and non-inducibility of NAT one day after application of 1-min light pulses

In rats maintained in LD 12:12 and then released into DD, the second day on DD the boundary between re-inducibility and non-inducibility of NAT by darkness after 1-min light pulses passes approximately through 0015 h (Illnerová and Vaněček 1984). When rats are presented with 1-min light pulses the first night and then they are released into DD, the second night the boundary between re-inducibility and non-inducibility may be phase-shifted. For example, in rats pulsed at 2200 h the first night, NAT is still inducible by darkness after a 1-min light pulse applied at 0100 h on the second night, but not after one at 0200 h. The boundary in these rats is thus about 0130 h and it is phase--delayed by 1.25 h relative to the boundary in unpulsed controls. In rats pulsed at 0300 h the first night, NAT is still inducible by darkness after a pulse at 2100 h the second night, but not after one at 2200 h. The boundary thus passes approximately through 2130 h and it is phase-advanced by 2.75 h relative to the boundary in unpulsed controls. Phase-shifts of the boundary between re-inducibility and non-inducibility of NAT one day after application of 1-min light pulses at different night times are summarized in the PRC (Fig. 4, C) (Illnerová and Vaněček 1984). When the pulses are presented before midnight the first night, the second night the boundaries between re-inducibility and non-inducibility of NAT after 1-min light pulses are phase--delayed relative to the boundary in unpulsed rats. When the pulses are presented after midnight the first night, the second night boundaries are phase-advanced. The PRC representing shifts of the boundary (Fig. 4, C) and the PRC representing shifts of the morning NAT decline (Fig. 4, B) are almost identical. Hence both PRCs may be manifestations of phase-shifts of the morning oscillator. Re-inducibility or non-inducibility of NAT after brief light pulses at night may thus be solely a function of the phase of M at the time of light pulse presentation. The boundary may indicate a certain phase of M, in which M begins to be sensitive to light and can be instantaneously phase-advanced by a light pulse to a phase when NAT activity is already low, i.e. the boundary may indicate the phase when M assumes its role in the synchronization of the NAT rhythm by light.

3.3 General discussion on the two-oscillator pacemaking system controlling the circadian NAT rhythm

Our results indicate that in the rat pineal gland the circadian NAT rhythm is controlled by a complex pacemaker consisting of at least two oscillators, the evening one, E, controlling the NAT rise and the morning one, M, controlling the NAT decline. E may be coupled to dusk, M to dawn. Both oscillators are coupled in a mutual phase-relation and interact with each other (Illnerová and Vaněček 1982 a,b). When a light pulse is presented in the first half of night, E may be instantaneously phase-delayed (Fig. 2). The next night and thereafter, the phase-delays are smaller than instantaneous phase-shifts of E, due to mutual interaction between E and M (Fig. 3, 4, A). The phase-delayed E may force M to phase-delay. The night when a pulse is applied before midnight, the morning NAT decline, which represents a phase of M, is phase-delayed only slightly (Illnerová and Vaněček 1982 a, 1984), however the next night M is already phase-delayed almost as much as E (Fig. 3, 4, B) and after four days phase-delays of E and M may be equal (Fig. 3). When a light pulse is presented after midnight, M may be instantaneously phase-advanced (Fig. 2, 4, C). The next night and thereafter, the phase-advances are smaller than instantaneous phase-shifts of M. One day after 1-min light pulses, E is not yet phase-advanced by the phase-advanced M (Fig. 3, 4, A); only after four days, E becomes to be phase-advanced too (Fig. 3) (Illnerová and Vaněček 1983). However, even at that time, the amount of the phase-advance of E is still smaller than that of M. Hence, E may be more stable and may have greater impact on M than vice versa.

4 PHASE-RELATION BETWEEN THE EVENING AND THE MORNING OSCILLATOR

4.1 Rhythms in NAT and melatonin under different photoperiods

The time when NAT activity in the rat pineal gland reaches the value of 3 nmol $mg^{-1}h^{-1}$ during its evening rise may be arbitrarily chosen as the phase-reference point for E. Similarly, the time when NAT activity declines to 3 nmol mg^{-1} h^{-1} during its decrease may be chosen as the phase-reference point for M. The time interval between both reference points indicates phase-relation between E and M showing at the same time duration of the period when NAT activity is equal or higher than 3 nmol mg^{-1} h^{-1}. Thus the phase-relation between both oscillators may determine the period of elevated night NAT and melatonin levels (Illnerová and Vaněček 1982 a,b). Light intruding in the late evening hours may phase-delay E, just as light intruding in the early morning hours may phase-advance M. Therefore, it is possible to infer that on long days the phase-relation between E and M and hence the duration of elevated nocturnal NAT and melatonin levels may be compressed and on short days it may be decompressed. It appears to be really so. In rats maintained under the artificial lighting regime LD 16:8 or in natural daylight in June, the period of elevated NAT levels is compress-

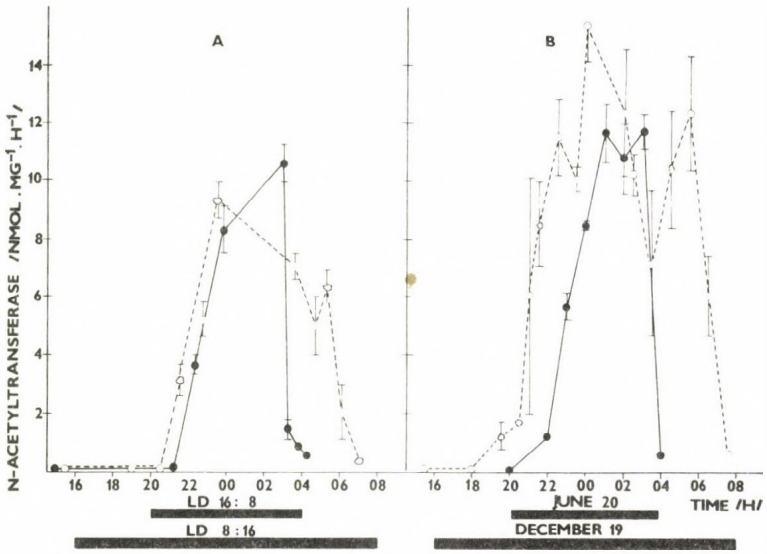

Fig. 5. The N-acetyltransferase rhythm under artificial light--dark regimes (left side of the figure, A) or in natural daylight (right side, B). Lines under abcissa indicate periods of darkness or periods between sunsets and sunrises, respectively. Filled circles, rats kept for 5 weeks in LD 16:8 (left side) or rats kept in natural daylight and killed on June 20 (right side). Open circles, rats kept for 5 weeks in LD 8:16 (left side) or rats kept in natural daylight and killed on December 19 (right side). Data are expressed as means ± SEM of four (left side) or three (right side) animals and were taken from Illnerová and Vaněček (1980).

ed as compared with the period under LD 8:16 or in natural daylight in December (Fig. 5) (Illnerová and Vaněček 1980, 1982b). The phase-relation between E and M is 5.1 h under LD 16:8, 8.5 h under LD 8:16, 5.3 h in June and 10.3 in December. The extension of the period proceeds mainly into the morning hours.

The period of elevated NAT and melatonin levels is longer under short than under long days also in other animals: in Djungarian hamsters (Hoffmann et al. 1981, Goldman et al. 1981, Illnerová et al. 1984), Syrian hamsters (Brainard et al. 1982), white-footed mice (Robert Lynch, personal communication), ewes (Rollag and Niswender 1976, Arendt et al. 1981) and chicken (Binkley et al. 1977). Hence patterns of melatonin release, namely the duration of nocturnal melatonin production and secretion, may provide animals with the information on day length (Hoffmann 1978, 1981, Hoffmann et al. 1981, Illnerová and Vaněček 1980, 1983, Goldman et al. 1981, Carter and Goldman 1983). When the period of elevated night melatonin levels is artificially prolonged on long days either by the administration of exogenous

melatonin to intact Syrian hamsters at a proper time (Tamarkin et al. 1976) or by the increase of endogenous melatonin after the beta-adrenergic agonist administration to male rats sensitized to short photoperiods (Vaněček and Illnerová 1983), physiological responses of animals are similar to the responses to short days. From this point of view the importance of brief light pulses at night is evident. For example, the phase-relation between E and M may be drastically reduced by a 1-min light pulse applied at 0300 h and the compression may persist not only for one day, but apparently for four days and even longer (Fig. 3) (Illnerová and Vaněček 1983). Consequently, the period of elevated melatonin levels may be shortened and the pulsed animals may receive wrong informations on day length.

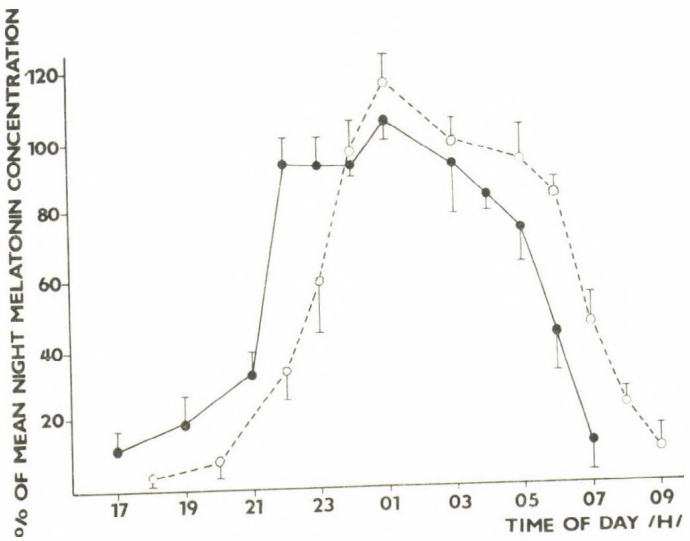

Fig. 6. Rhythm in plasma melatonin concentration of the urbanised man in July (filled circles) and January (open circles). Five healthy volunteers were sampled between July 4 and 5, and again these five persons plus two other volunteers were sampled between January 13 and 14. Melatonin in plasma was measured by direct radioimmunoassay (Fraser et al. 1983) with the antiserum, batch 704-189, provided kindly by dr. J. Arendt, via Guildhay Antisera, Department of Biochemistry, University of Surrey. For each individual, the mean night melatonin concentration was calculated from five highest values and it was held as 100 %. Individual patterns of melatonin rhythms were expressed in % of the mean night value. Points are means of 5 or 7 persons ± SEM.

It seems that humans, at least urbanised persons, might be an exception among mammals, as they do not respond to short days in winter by extension of the period of elevated plasma melatonin

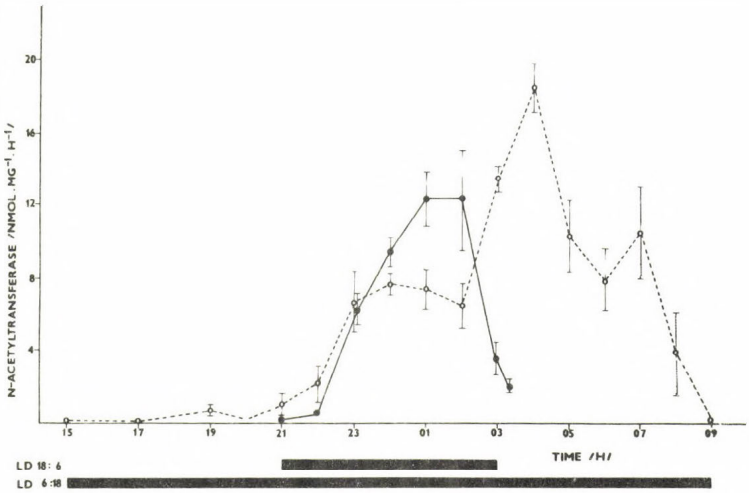

Fig. 7. The N-acetyltransferase rhythm in rats maintained for 4 weeks under LD 18:6 (closed circles) or under LD 6:18 (open circles). Lines under abcissa indicate periods of darkness. Data are expressed as means ± SEM of 4 animals.

levels. In January as in July, this period is about 8 h, however in January the whole rhythm is phase-delayed by 1.2 to 1.5 h relative to the pattern in July (the values are read from Fig. 6 at the level of 60 % of mean night melatonin concentrations). Nevertheless, it is necessary to bear in mind that urbanised persons might experience about 12 h of uninterrupted light of lesser intensity than is the necessary intensity for suppression of the high night melatonin production (Lewy et al. 1980). It cannot be excluded that in rural men patterns of melatonin rhythm might be different in summer and in winter.

4.2 Control of the NAT rhythm under extremely long and short photoperiods

When rats are maintained under LD 18:6, the period of elevated night NAT is about 4.6 h, when rats are maintained under LD 6:18, the period is about 10.1 h (Fig. 7). Even under such an extremely long photoperiod of 18 h of light per day, the NAT rhythm is synchronized. The morning NAT decline occurs spontaneously before lights-on. Under the extremely short photoperiod of 6 h of light per day, the NAT rhythm extends into the morning hours and the evening rise occurs only six hours after lights--off. Under the long photoperiod, the phase-relation between E and M is apparently compressed to a high degree, under the short photoperiod the phase relation may be completely decompressed. The strength of the mutual interaction between E and M may de-

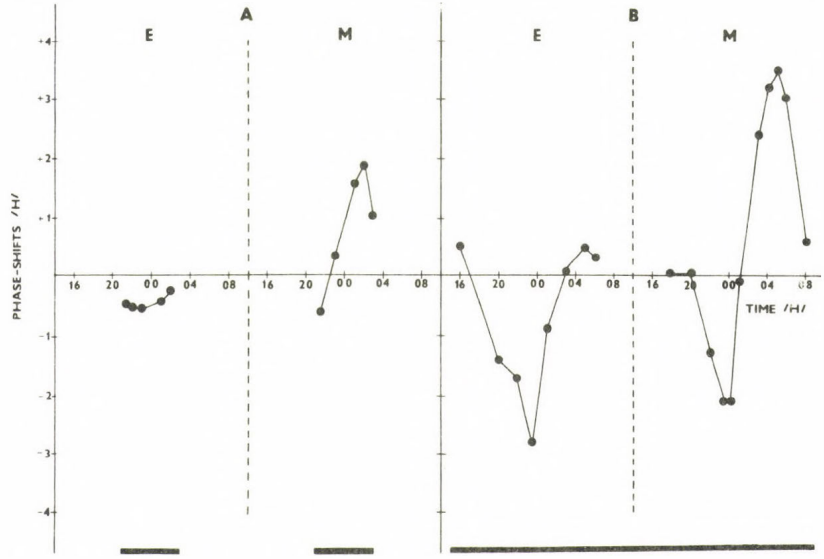

Fig. 8. Phase-response curves of the evening NAT rise (E) and of the morning N-acetyltransferase decline (M) one day after application of 1-min light pulses to rats maintained for four weeks under LD 18:6 (A) or under LD 6:18 (B). Values of phase--stifts were read from the graphic illustration of evening N-acetyltransferase rises and of morning N-acetyltransferase declines in pulsed rats and in unpulsed controls (not shown). Abcissa denotes time of pulse presentation. Phase-delays are expressed by the sign -, phase-advances by the sign +.

pend on the phase-relation between them (Pittendrigh and Daan 1976, Illnerová and Vaněček 1982a,b). The more the phase-relation is compressed, the more the interaction between E and M may affect separate phase-shifts of E and M and thus influence the net phase-shifts of the coupled system. Hence the resultant phase-shifts of E and M one day after application of brief light pulses at night might be affected more by the interaction between E and M under the extremely long photoperiod than under the short photoperiod. It seems to be really so (Fig. 8). Rats maintained either under LD 18:6 or LD 6:18 were exposed to 1-min light pulses at different night times or they were left unpulsed, then they were released into DD and the next night the evening NAT rises and the morning NAT declines were determined. Phase-shifts of the evening NAT rise and of the morning NAT decline of the pulsed rats relative to the patterns in the unpulsed controls under long days (Fig. 8, A) and under short days (Fig. 8, B) are summarized in PRCs. Under LD 18:6, in the compressed state of the phase-relation between E and M, phase--shifts of E and M one day after 1-min light pulses are smaller than shifts under LD 6:18 or under LD 12:12 (Fig. 4, A,B), due to a stronger interaction between E and M. Light pulses at

2300 h and at 0100 h slightly phase-delay E, but at the same time they phase-advance M. This observation may indicate that under a very long photoperiod a pulse applied around midnight may interfere with both oscillators and hence spheres of E and M may overlap. Under LD 6:18, phase-shifts of E and M one day after 1-min light pulses are larger than shifts under LD 18:6 or under LD 12:12 (Fig. 4, A,B), due to a weaker interaction between E and M. Obviously, shifts of the NAT rhythm may vary depending on the lighting regime under which animals are maintained. Under LD 18:6, a 1-min light pulse applied within 1 h after lights-off phase-delays E the next day, the pulse applied within 1 h before lights-on phase-advances M. Under LD 6:18, a light pulse applied within 1 h after lights-off does not phase--delay E, however a pulse applied 1 h before lights-on still phase-advances M. Hence it may be inferred that under long days the NAT rhythm is synchronized by evening as well as by morning light, while under short days the rhythm may be synchronized only by morning light and the phase-relation between E and M may be stable.

5 CONCLUSIONS

The circadian rhythm of NAT activity in pineal gland of rats and Djungarian hamsters drives the rhythm in melatonin production and regulates the duration of the period of elevated nocturnal melatonin levels. Our results indicate that in rats the NAT rhythm may be driven by a complex pacemaker consisting of at least two oscillators, the evening one, controlling the NAT rise and coupled to dusk and the morning one, controlling the NAT decline and coupled to dawn. Both oscillators are conpled in a mutual phase-relation and interact with each other. The phase-relation may determine the period of high night NAT activity. Under long photoperiods, the phase-relation between the evening and the morning oscillator is compressed and the period of elevated nocturnal NAT and melatonin levels is short. Under short photoperiods the phase-relation is decompressed and hence the duration of elevated night NAT and melatonin levels is long. Brief light pulses at night may also compress the phase-relation between both oscillators. On long days, the NAT rhythm may be synchronized by evening as well as by morning light, on short days only by morning light.

REFERENCES

Arendt, J., Symons, A. M. and Laud, C. (1981) Pineal function in the sheep: evidence for a possible mechanism mediating seasonal reproductive activity. Experientia 37 : 584-586

Axelrod, J. and Weissbach, H. (1960) Enzymatic O-methylation of N-acetylserotonin to melatonin. Science 131 : 1312-1313

Binkley, S., Klein, D. C. and Weller, J. (1974) Dark induced increase in pineal serotonin N-acetyltransferase activity: A refractory period. Experientia 29 : 1339-1340.

Binkley, S., Stephens, J. L., Riebman, J. B. and Reilly, K. B. (1977) Regulation of pineal rhythms in chickens. Photoperiod and dark-time sensitivity. Gen. comp. Endocr. 32 : 411-416

Brainard, G. C., Petterborg, L. J., Richardson, B. A. and Reiter, R. J. (1982) Pineal melatonin in Syrian hamsters: circadian and seasonal rhythms in animals maintained under laboratory and natural conditions. Neuroendocrinology 35 : 342-348

Brownstein, M. and Axelrod, J. (1974) Pineal gland. 24-hour rhythm in norepinephrine turnover. Science 184 :163-164

Carter, D. S. and Goldman, B. D. (1983) Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungorus sungorus). Duration is the critical parameter. Endocrinology 113 : 1261-1267

Deguchi, T. and Axelrod, J. (1972) Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the beta-adrenergic receptor. Proc. natn. Acad. Sci. USA 69 :2547-2550

Frazer, S., Cowen, P. and Franklin, M. (1983) Direct radioimmunoassay for melatonin in plasma. Clin. Chem. 29 : 396-401

Goldman, B. D., Hall, V., Hollister, C., Reppert, S., Roychoudhury, P., Yellon, S. and Tamarkin, L. (1981) Diurnal changes in pineal melatonin content in four rodent species: relationship to photoperiodism. Biol. Reprod. 24 : 778-783

Hoffmann, K. (1978) Photoperiodic mechanisms in hamsters. The participation of the pineal gland. In: Environmental Endocrinology, Assenmacher, J. and Farner, D. S., editors, Springer, Berlin, pp. 94-102

Hoffmann, K. (1981) The role of the pineal gland in the photoperiodic control of seasonal cycles in hamsters. In: Biological Clocks in Seasonal Reproductive Cycles, Follet, B. K. and Follet, D. E., editors, John Weight and Sons, Bristol, pp. 237-250

Hoffman, K., Illnerová, H. and Vaněček, J. (1981) Effect of photoperiod and of one minute light at night-time on the pineal rhythm in N-acetyltransferase activity in the Djungarian hamster Phodopus sungorus. Biol. Reprod. 24 : 551-556

Illnerová, H. (1971) Effect of light on the serotonin content of the pineal gland. Life Sci. 10, Part I : 955-960

Illnerová, H. (1974) The effect of darkness and pargyline on the activity of serotonin N-acetyltransferase in the rat epiphysis. Neuroendocrinology 16 : 202-211

Illnerová, H. and Vaněček, J. (1979) Response of rat pineal serotonin N-acetyltransferase to one min light pulse at different night times. Brain Res. 167 : 431-434

Illnerová, H. and Vaněček, J. (1980) Pineal rhythm in N-acetyltransferase activity in rats under different artificial photoperiods and in natural daylight in the course of a year. Neuroendocrinology 31 : 321-326

Illnerová, H. and Vaněček, J. (1982a) Two-oscillator structure of the pacemaker controlling the circadian rhythm of N-acetyltransferase in the rat pineal gland. J. Comp. Physiol. A145 : 539-548

Illnerová, H. and Vaněček, J. (1982b) Complex control of the circadian rhythm in N-acetyltransferase activity in the rat pineal gland.. In Vertebrate Circadian Systems, Aschoff, J., Daan, S. and Groos, G., editors, Springer-Verlag, Berlin, Heidelberg, pp. 285-296

Illnerová, H. and Vaněček, J. (1983) Extension of the rat pineal N-acetyltransferase rhythm in continuous darkness and on short photoperiod. Brain Res. 261 : 176-179

Illnerová, H. and Vaněček, J. (1984) Circadian rhythm in inducibility of rat pineal N-acetyltransferase after brief light pulses at night: control by a morning oscillator. J. Comp. Physiol. A, in press

Illnerová, H., Backström, M. Sääf, J., Wetterberg, L. and Vangbo, B. (1978) Melatonin in the rat pineal gland and serum; rapid parallel decline after light exposure at night. Neurosci. Lett. 3 : 189-193

Illnerová, H., Vaněček, J., Křeček, J., Wetterberg, L. and Sääf, J. (1979) Effect of one minute exposure to light at night on rat pineal serotonin N-acetyltransferase and melatonin. J. Neurochem. 32 : 673-675

Illnerová, H., Vaněček, J. and Hoffmann, K. (1983) Regulation of the pineal melatonin concentration in the rat (Rattus norvegicus) and in the Djungarian hamster (Phadopus sungorus). Comp. Biochem. Physiol. 74 A : 155-159

Illnerová, H., Hoffmann, K. and Vaněček, J. (1984) Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster Phodopus sungorus. Neuroendocrinology 38 : 226-231

Klein, D. C. (1979) Circadian rhythms in the pineal gland. In: Endocrine Rhythms, Krieger, D. T., editor, Raven Press, New York, pp. 203-223

Klein, D. C. and Berg, G. R. (1970) Pineal gland: stimulation of melatonin production by norepinephrine involves cyclic AMP-mediated stimulation of N-acetyltransferase. Adv. Biochem. Psychopharmacol. 3 : 241-263

Klein, D. C. and Moore, R. Y. (1979) Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res. 174: 245-262

Klein, D. C. and Weller, J. E. (1970) Indole metabolism in the pineal gland. A circadian rhythm in N-acetyltransferase activity. Science 169 : 1093-1095

Klein, D. C. and Weller, J. E. (1972) Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. Science 177 : 532-533

Klein, D. C., Weller, J. E. and Moore, R. J. (1971) Melatonin metabolism. Neural regulation of pineal serotonin: acetylcoenzyme A N-acetyltransferase activity. Proc. Natl. Acad. Sci. USA 68 : 3107-3110

Klein, D. C., Sugden, D. and Weller, J. E. (1983) Postsynaptic alpha-adrenergic receptors potentiate the beta-adrenergic stimulation of pineal serotonin N-acetyltransferase. Proc. Natl. Acad. Sci. USA 80 : 599-603

Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. and Markey, S. P. (1980) Light suppresses melatonin secretion in humans. Science 210 :1267-1269

Perlow, M. J., Reppert, S. M., Boyar, R. M. and Klein, D. C. (1981) Daily rhythms in cortisol and melatonin in primate cerebrospinal fluid. Neuroendocrinology 32 : 193-196

Pittendrigh, C. S. and Daan, S. (1976) A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker

structure: A clock for all seasons. J. Comp. Physiol. A 106 : 333-355
Quay, W. B. (1967) Lack of rhythm and effect of darkness in rat pineal content of N-acetylserotonin-O-methyltransferase. Physiologist 10 : 286
Ralph, C. L., Hull, D., Lynch, H. J. and Hedlund, L. (1971) A melatonin rhythm persists in rat pineals in darkness. Endocrinology 89 : 1361-1366
Ralph, C. L., Binkley, S. E., MacBride, S. and Klein, D. C. (1975) Regulation of pineal rhythms in chickens: Effect of blinding, constant light and superior cervical ganglionectomy. Endocrinology 97 : 1373-1379
Reiter, R. J., Steinlechner, S., Richardson, B. A. and King, T. S. (1983) Differential response of pineal melatonin levels to light at night in laboratory-raised and wild-captured 13-lined ground squirrels (Spermophilus tridecemlineatus). Life Sci. 32 : 2625-2629
Rollag, M. D. and Niswender, G. D. (1976) Radioimmunoassay of serum concentration of melatonin in sheep exposed to different lighting regimens. Endocrinology 98 : 482-488
Tamarkin, L., Westrom, W. K., Hamill, A. I. and Goldman, G. C. (1976) Effect of melatonin on the reproductive systems of male and female Syrian hamsters: A diurnal rhythm of sensitivity to melatonin. Endocrinology 99: 1534-1541
Tamarkin, L., Reppert, S. M. and Klein, D. C. (1979) Regulation of pineal melatonin in the Syrian hamster. Endocrinology 104 : 385-389
Vaněček, J. and Illnerová, H. (1979) Changes of a rhythm in rat pineal serotonin N-acetyltransferase following a one-minute light pulse at night. In: The Pineal Gland of Vertebrates Including Man, Arriens Kappers, J. and Pévet, P., editors, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 245-248
Vaněček, J. and Illnerová, H. (1980) Some characteristics of the night N-acetyltransferase in the rat pineal gland. J. Neurochem. 35 : 1455-1457
Vaněček, J. and Illnerová, H. (1982a) Effect of light at night on the pineal rhythm in N-acetyltransferase activity in the Syrian hamster Mesocricetus auratus. Experientia 38 : 513-514
Vaněček, J. and Illnerová, H. (1982b) Night pineal N-acetyltransferase activity in rats exposed to white or red light pulses of various intensity and duration. Experientia 38 : 1318-1320
Vaněček, J. and Illnerová, H. (1983) The effect of daily evening isoproterenol administration on reproductive organ growth in male rats treated neonatally with testosterone propionate. Experientia 39 : 332-333
Weissbach, H., Redfield, B. G. and Axelrod, J. (1961) The enzymatic acetylation of serotonin and other naturally occurring amines. Biochim. Biophys. Acta 54 : 190-192
Wilkinson, M., Arendt, J., Bradtke, J. and de Ziegler, D. (1977) Determination of a dark-induced increase in pineal N-acetyltransferase activity and simultaneous radioimmunoassay of melatonin in pineal, serum and pituitary tissue of the male rat. J. Endocrinol. 72 : 243-244

Wurtman, R. J. and Ozaki, J. (1978) Physiological control of melatonin synthesis and secretion: Mechanisms generating rhythms in melatonin, methoxytryptophol, and arginine vasotocine levels and effects on the pineal of endogenous catecholamines, the estrous cycle and environmental light. J. Neural Transmission, Suppl. 13 : 59-70

The Pineal Gland
Current State of Pineal Research
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FACTORS INFLUENCING MELATONIN SECRETION IN SHEEP

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In ovines, as in an ever-increasing number of photoperiodic species, it has become evident that the presence of an intact pineal gland is essential for the recognition of changing daylength, particularly in respect of reproductive responses. The work of Lincoln (1979) and Bittman et al (1983a) has shown that denervation of the gland or pinealectomy in rams and pinealectomy in ewes effectively blinds the animal to artificial photoperiod change. Pinealectomy in rams results in desynchronisation of the reproductive cycle with the annual cycle under natural photoperiodic conditions (Lincoln and Forbes, 1984).

Melatonin appears to be the major effector molecule for pineal-mediated effects in ovines, however before it became clear that either the pineal, or melatonin, were of importance, a number of groups began to define the characteristics of melatonin secretion in this species.

Work in the rat originally underlined the importance of light and darkness in the control of melatonin synthesis and excretion by the pineal and suggested that it might act as an information molecule with regard to the light-dark phase (Axelrod, 1974). The development of radioimmunoassay (RIA) technology for measurement of melatonin in body fluids (for a review see Arendt, 1978) provided the tools for the subsequent close definition of circulating levels.

Rollag and Niswender et al (1976) and Kennaway et al (1977) provided the first evidence that melatonin has a circadian rhythm in ewes, high values being found during the dark-phase as in all other species. The night-time rise is suppressed by continuous light, but the rhythm continues in constant darkness (Rollag and Niswender, 1976) and evidence from this and other studies shows that it is endogenous (Lincoln and Almeida, 1981) and entrained by the light-dark cycle. There is, so far one exception to this rule: in photorefractory rams, the melatonin rhythm can become disorganised and appears to escape from light-dark control (Almedia and Lincoln, 1984). The mechanism of escape is unknown but of very considerable interest.

Details of the central neural pathways controlling melatonin synthesis are not known in the sheep, however it is probable that the retina - retino-hypothalamic projection - suprachiasmatic nucleus route, substantially delineated in the rat (Moore and Klein, 1974) is also present in the sheep. The suprachiasmatic nucleus (SCN) may be the major central rhythm generator, however

it is dangerous to extrapolate across species, especially as in the monkey for example, the SCN does not have as important a role as in the rat (Reppert et al, 1981). Clearly, from the work of Lincoln et al (1981) the sympathetic innervation of the gland is essential for rhythmic melatonin secretion. Following sympathetic denervation (Lincoln et al, 1981) or pinealectomy (Arendt et al. 1980: Bittman et al. 1983b), the melatonin rhythm is lost and indeed following pinealectomy, serum values fall to undetectable levels by some, but not all assays (Arendt et al. 1980). The route of secretion of melatonin in the ewe appears to be primarily into the circulation (Rollag et al. 1978a) even though the close juxtaposition of the third ventricle to the pineal makes secretion into the CSF a possibility.

The 24 hour secretion pattern of melatonin in ovines is probably more complicated than a square wave, or a simple sine wave. Several authors report multiple secretory episodes (Arendt et al. 1981: Kennaway et al. 1983; Rollag et al. 1978a). Our work has consistently shown the presence of two, and occasionally more, secretory episodes during the dark-phase with sufficient length of night. In addition we have found small peaks in the mid-light phase in both March and October (Arendt, 1979). Analysis of the pattern of melatonin secretion in different natural photoperiod lengths showed that, by ANOVA, significant effects of time of day, time of year and number of hours after sunset and sunrise were found ($p<.01$ - $p<.001$, Arendt and Symons, 1981), suggesting that both dawn and dusk are of importance in the entrainment of secretory rhythms. In artificial photoperiod (12L12D) bimodal secretory patterns were also found in castrated rams (Arendt and Forbes, unpublished). In the rat, Illnerova et al (1982) have provided convincing evidence for a two-oscillator (dawn and dusk) control of melatonin secretion and it is possible that a similar model is applicable in sheep. The experiments required to investigate the model are lengthy and cumbersome in ovines - early work by Brinklow et al (1984) does however show that skeleton photoperiod, with a one-hour light flash 17 hours after dawn (7L,10D, 1L, 6D) is sufficient to suppress melatonin secretion during the remaining dark phase, comparable to the one-minute flash used in the rat by Illnerova et al (1982), thus opening the way for a detailed investigation of the nature of the photoperiodic and oscillatory control of melatonin secretion in sheep. Lincoln and Almeida (1981) have shown, moreoever, that resonance light cycles of 8L,40D are sufficient to entrain melatonin secretion in rams.

Although there is no real consensus in the detailed description of melatonin secretory patterns, all authors have found that the duration of high melatonin secretion in sheep reflects the length of the dark-phase, be it artificial or natural (Rollag et al. 1978b; Arendt et al. 1981; Kennaway et al. 1983; Bittman et al. 1983b; Bittman and Karsch, 1984). This observation is not necessarily true of all photoperiodic species. Nevertheless it has stimulated a number of important experiments i.e. the generation of artificial melatonin rhythms in serum corresponding to long or short dark and subsequent observations of reproductive state.

In the intact ewe, in summer light, late afternoon feeding of melatonin to generate a Winter plasma profile results in early onset of oestrus cycles and a dramatic drop (Kennaway et al. 1982) and change in the pattern of secretion of prolactin (Arendt et al. 1983; Symons et al. 1983). In addition to the obvious benefit of manipulating the time of lambing, this manoevre results apparently in an increased ovulation rate (Kennaway et al. 1984), for reasons unknown.

In pinealectomised ewes, creation of artifical long or short dark melatonin plasma profiles results in the appropriate reproductive response, assuming the animals reads the profiles as long or short night respectively (Bitman and Karsch, 1984) and regardless of the prevailing photoperiod. That the duration of melatonin secretion can be a signal for photoperiod length is clear, however, this cannot be the entire explanation: photoperiod length is the same twice a year and in the one case is followed or accompanied by reproductive quiescence and in the other by resurgence. Moreoever implants of melatonin, giving continuous high plasma levels (Kennaway et al. 1982) can advance the whole seasonal cycle of reproductive competence, providing the animals have been previously exposed to long days (Lincoln et al. 1984). The story is by no means complete and the next few years should be see major advances in our understanding of the mechanisms of action of melatonin.

Returning to the question of the control of melatonin secretion, whilst the light-dark phase is of overriding importance, gonadal steroids may exert a tonic inhibitory influence. Ovariectomised ewes, 16 and 58 days after surgery have significantly higher plasma melatonin than intact controls (Arendt et al. 1983) although the 24-hour rhythm is similar in both groups. In time, however, these differences lose their significance hence compensatory mechanisms must be activated (Arendt and Symons, unpublished results).

Feedback effects of melatonin on its own secretion are unlikely to be of major importance in ovines: in the presence of melatonin implants, a normal night-time rise is seen, superimposed on continuously high plasma levels (Kennaway et al. 1982). Likewise feeding of melatonin in the late afternoon does not obviously modify the endogenous secretion, and in a small number of animals (3) the presence of circulating melatonin antibodies (assuming that most biologically active melatonin was bound) does not appear to affect the 24-hour rhythm of secretion (Arendt and Symons, unpublished) although in the latter case clearly conclusions cannot be drawn from such a small group of animals.

The pharmacology of the sheep pineal is little understood. One preliminary report described the suppression of the night-time melatonin rise by prazosin, an α-adrenergic antagonist (Klein et al. 1983). In our hands, in preliminary experiments, neither prazosin (1-4mg i.v., or 10-20mg per os) or atenolol, a specific β_1-peripheral adrenergic antagonist (100mg per os) had consistent effects on the night-time rise or 24 hour pattern of plasma melatonin when given acutely, 30 minutes before dusk, to oestrus ewes (English, Arendt and Symons, unpublished). It would be of interest to shorten the duration of melatonin secretion in long dark in intact ewes, in order to attempt the induction of long-day effects, hence for this reason and also in the interest of defining the neural input to the sheep pineal, this area is worth pursuing. Precursor availability is probably an important consideration in sheep, in that the administration of 5-hydroxytryptophan during the day is able to increase plasma melatonin (Namboodiri et al. 1983).

There are multiple possibilities for modulation of pineal secretion. Endocrine and other factors may modify the central oscillator (s) governing pineal secretion, their efferent neural pathways, and may have direct effects on neural stimulation, message translation and synthetic machinery of the pineal. The sheep is an ideal species in which to study the end-point: peripheral secretion.

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REFERENCES

1. Almeida, OFX, Lincoln, GA (1984). Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. Biol of Reprod 30; 143-159.

2. Arendt, J (1978). Melatonin assays in body fluids. J Neural Trans 13; 265-278.

3. Arendt J (1979). Radioimmunoassayable melatonin: circulating patterns in man and sheep. Prog Brain Res 52; 249-258.

4. Arendt, J Brown WB, Forbes JM, Marston A (1980). Effect of pinealectomy on immunoassayable melatonin in sheep. J Endocrinol 85; 1-2p.

5. Arendt J, Symons AM (1981). In: Biological clocks in seasonal reproductive cycles, Eds: Follett BK, Follett DE. Bristol: Wright;p276.

6. Arendt J, Symons AM, Laud CA (1981a). Pineal function in the sheep: evidence for a possible mechanism mediating seasonal reproductive activity. Experientia 37: 584-586.

7. Arendt J, Symons, AM, Laud CA (1981b). Pineal function and photoperiod in the ewe. In: Photoperiodism and reproduction; Eds: Ortavant, R, Pelletier J, Ravault JP. INRA Publications, Nouzilly, France, 219-229.

8. Arendt J, Symons AM, Laud CA, Pryde SJ (1983a). Melatonin can induce early onset of the breeding season in ewes. J Endocrinol 97; 395-400.

9. Arendt J, Laud CA, Symons AM (1983b). Plasma melatonin increases in ewes following ovariectomy. J Reprod Fert 68; 213-218.

10. Axelrod J (1974). The pineal gland: a neurochemical transducer. Science 184; 1341-1348.

11. Bittman EL, Karsch FJ, Hopkins JW (1983a) Role of the pineal gland in ovine photoperiodism: regulation of seasonal breeding and negative feedback effects of estradiol upon luteinising hormone secretion. Endocrinol 113; 329-336.

12. Bittman EL, Dempsey J, Karsch FJ (1983b). Pineal melatonin secretion drives the reproductive response to daylength in the ewe. Endocrinol 113; 2276-2283.

13. Bittman EL, Karsch, FJ (1984). Nightly duration of pineal melatonin secretion determines the reproductive response to inhibitory daylength in the ewe. Biol Reprod 30; 585-593.

14. Brinklow BR, Forbes MJ, Rodway RG (1984). Melatonin in the plasma of growing sheep subjected to short and skeleton long photoperiods. Experientia (in press).

15. Illnerova H, Vanecek J (1982). Complex control of the circadian rhythm in N-acetyltransferase activity in the rat pineal gland. In: Vertebrate circadian systems, Eds: Aschoff J, Daan S, Groos G; Springer Verlag, Berlin; 285-296.

16. Kennaway DJ, Frith RC, Phillipou G, Matthews CD, Seamark RF (1977). A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas-chromatography mass-spectrometry. Endocrinol 101; 119-127.

17. Kennaway DJ, Gilmore TA, Seamark RF (1982). Effect of melatonin feeding on serum prolactin and gonadotrophin levels and the onset of seasonal estrous cyclicity in sheep. Endocrinol 110; 1766-1722.

18. Kennaway DJ, Gilmore TA, Seamark RF (1982). Effects of melatonin implants on the circadian rhythm of plasma melatonin and prolactin in sheep. Endocrinol 110; 2186-2188.

19. Kennaway DJ, Sanford LM, Godfrey B, Friesen HG (1983). Patterns of progesterone, melatonin and prolactin secretion in ewes maintained in four different photoperiods. J Endocrinol 97; 229-242.

20. Kennaway DJ, Dunstan EA, Gilmore TA, Seamark RF (1984). Melatonin feeding increases ovulation rate in ewes. VII International Congress of Endocrinology, Abstr. No. 1021.

21. Klein DC, Namboodiri MAA, Sugden D (1983). New in vivo approaches to studying melatonin production. XIV Acta Endocrinologica Congress, Satellite Symposium on the pineal. Abstract No. 8.

22. Lincoln GA (1979). Photoperiodic control of seasonal breeding in the ram: participation of the cranial sympathetic nervous system. J Endocrinol 82; 135-147.

23. Lincoln GA, Almeida OFX, Arendt J (1981). Role of melatonin and circadian rhythms in seasonal reproduction in rams. J Reprod Fert 30; 23-31.

24. Lincoln GA, Almeida OFX (1981). Melatonin and the seasonal photoperiodic response in sheep. In: Photoperiodism and Reproduction, Eds. Ortavant R, Pelletier J, Ravault JP, INRA Publications, Nouzilly, France 231-251.

25. Lincoln GA, Forbes JM (1984). Seasonal physiology of pinealectomised rams. Society for the Study of Fertility, Abstract No. 33.

26. Moore RY, Klein DC (1974). Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase. Brain Res 71; 17-33.

27. Namboodiri MAA, Sugden D, Klein DC, Mefford IN (1983). 5-hydroxytryptophan elevates serum melatonin. Science 221; 659-661.

28. Reppert SM, Perlow MJ, Ungerleider LG, Mishkin M, Tamarkin L, Orloff D, Hoffman HJ, Klein DC (1981). Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the Rhesus monkey.. J Neurosci 1; 1414-1425.

29. Rollag MD, Niswender GD (1976). Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinol 98; 482-489.

30. Rollag MD, Morgan RJ, Niswender GD (1978a). Route of melatonin secretion in sheep. Endocrinol 102; 1-8.

31. Rollag MD, O'Callaghan PL, Niswender GD (1978b). Serum melatonin concentrations during different stages of the annual reproductive cycle in ewes. Biol Reprod 18; 279-285.

31. Symons AM, Arendt J, Laud CA (1983). Melatonin feeding decreases prolactin levels in the ewe. J Endocrinol 99; 41-46.

PINEAL AND ENVIRONMENT, WITH SPECIAL REFERENCE TO SEASONAL REPRODUCTION

The Pineal Gland
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5-METHOXYINDOLES, PINEAL, AND SEASONAL REPRODUCTION - A NEW APPROACH

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The habitat of most organisms is subject to pronounced seasonal fluctuations. Literally all physical environmental factors important to an organism, such as temperature, daylength and rainfall, vary with the season. Animals have therefore to adapt themselves to these more or less large variations of the environment, depending upon the latitude, but also the altitude. To ensure the survival of the species they have in particular to synchronize their reproductive activity with the period of the year when the conditions of life are optimal for the offspring.

The reproductive cycle is essentially a matter of temporal organization in relation to environmental resources. Changes in the biometereological conditions signal the endocrine system which responds by an adaptive alteration of its physiological status in anticipation of the upcoming season.

Since ancient times it is known that there are several seasonal and/or environmental variables on which animals can rely to cue changes in their sexual status. Daylength, temperature, rainfall, food availability, etc. can be considered but, depending on the biotope, some are more accurate predictors of the forthcoming season than others. In temperate and arctic zones, for example, in which drastic climatic conditions impose a precise sexual cycle, one of the most regularly changing phenomena is the daily change in daylength (photoperiod), and the role of this factor as primary productive information in the control of the annual cycle has been well established (for a review see Perry and Rowlands, 1972). Other environmental factors, however, should also be considered. In amphibians and reptiles, thermoperiodism is generally dominant to photoperiodism (for a review see Lofts, 1974). In the tropical and equatorial zones of the world, where the fauna is immensely rich in species, more irregularly recurring external stimuli such as e.g. rainfall are equally precise and used for timing seasonal functions. Moreover, in the field there is always a co-variation of all these potential proximate factors. For example, in temperate zones in autumn the decrease in photoperiod is always associated with a decrease in mean temperature and food availability, and it is evident that interactions exist between the effects of all these factors.

The precise mechanism by which the central nervous system is able to perceive, differentiate, and ultimately integrate this complex of various stimuli has not yet completely been identified. However, an indole derivative — melatonin — and a gland — the pineal — are known to be greatly implicated in such mechanism (for a review see Klein, 1982).

The present review is an attempt to evaluate the role of the pineal and also that of other organs which, like the pineal gland, synthesize indole-derivatives in the integration of environmental information.

I. RELATIONSHIPS BETWEEN PINEAL AND ENVIRONMENTAL CONDITIONS

In comparison with all other endocrine glands, the pineal is characterized by the fact that, according to the species, an extraordinarly large variation in size is observed. These interspecies differences in pineal volume, which are far greater than variations stemming from daily or seasonal cycles, have a primarily genetic basis (Pévet, 1983a), which means that the difference in size is of physiological significance.

The first experimental proofs that the photoperiod-induced gonadal changes in mammals are pineal-dependent were produced by Czyba *et al.* (1964) and by Hoffman and Reiter (1965). These authors observed that pinealectomy prevents in the golden hamster the gonadal regression normally brought about by blinding or by experimental or natural short photoperiod. Since that time these results have been confirmed or extended in numerous other species, and the pineal is now recognized as an active endocrine gland which serves as a critical link between photoperiodic information and the neuroendocrine system. (For a review, see Goldman, 1983).

This concept is supported by the morphologic analysis of Ralph (1975) who, relating pineal size to geography, suggested that animals which inhabit tropical regions where the annual photoperiodic changes are small, are more apt to have a small pineal body, or none at all, while large pineal glands are found more often in animals living at high latitudes where the annual changes in daylight are considerable. Such interpretation, however, does not explain why numerous mammals and non-mammalian vertebrates living in equatorial/tropical zones (e.g. camel (Ralph, 1975), fruit bat (Pévet, 1983a), golden mole (Pévet and Kuypers, 1978), Malaysian rat (Pévet and Yadav, 1980), tenrec (Pévet, 1983a), green sea turtle and other equatorial/tropical turtles (Owens *et al.*, 1978; Vivien-Roels and Pévet, 1983)) possess a large and active pineal organ. These observations might be explained by considering not the changes in environmental lighting conditions, but the changes in environmental conditions in general. In equatorial/tropical zones, which are essentially characterized by the fact that the monthly mean temperature does not show much seasonal fluctuation (DeLany and Happolid, 1979), the pressure of the environment is not very strong if compared to that of temperate and, especial-

ly, arctic zones. This is particularly true in the equatorial wet regions where not only mean temperature but also rainfall and consequently the availability of food, remain high and constant throughout the year. Most species characterized by an absence of the pineal (anteater, sloth, armadillo, pangolin, crocodile, electric ray; ref. in Pévet, 1983a, and Vivien-Roels and Pévet, 1983) or by a very small one (rhinoceros, elephant, dugong, red kangaroo; ref. in Pévet, 1983a) live in tropical/equatorial zones. However, except for the equatorial wet forests, most of the tropical zones are characterized by a seasonal rhythm in annual rainfall, and many animal species present an annual sexual cycle more or less well synchronized with the rainfall (DeLany and Happolid, 1979), and in desert zones the conditions of life are difficult for animal species.This would explain the presence of a large and active pineal in these animals. In higher latitude temperate and arctic zones, variations in climatologic conditions, especially the annual variations in temperature and food availability, are large. The animals have to adapt themselves to these variations of the environment, the magnitude of which depends on the altitude as well as the latitude. Animals living in these regions, such as the sea lion, seal, walrus, Weddell and elephant seals, and some cervidae (ref. in Ralph, 1975; and Pévet, 1983a) all have a very large pineal.

In such an approach, the increase in pineal size at high latitudes clearly demonstrated in rodents (Legait *et al.*, 1976; Quay, 1980) should be related to the increase in critical value of neuroendocrine transduction for timing and exact phase tuning of biological rhythms. So it would be the correlations between pineal size, not with latitude but with latitude-related biotopes (e.g. equatorial forest, savanna, desert zones, arctic and holarctic zones combined with zonation in altitude), in which the influence of environmental conditions and probably the nature of the dominant environmental cue differ, which should be considered.

In short, the more the environmental conditions of life are difficult to the animal, the more will synchronization of the reproductive activity with the environment be indispensable: the more so would the pineal be important.

This raises the question of how the pineal integrates all of this information, and that of the nature of the hormonal compounds which act on the gonadal axis. Two groups of compounds have been identified and proposed to be responsible for the endocrine capabilities of the pineal gland: indoleamines — especially 5-methoxyindoles — and peptidic/proteic compounds.

II. PROTEIC OR PEPTIDIC PINEAL SUBSTANCES: THEIR ROLE

Since 1920 it has been constantly demonstrated that proteic or peptidic fractions extracted from different pineal tissues can induce, under defined experimental conditions, an inhibition or stimulation of the gonadal activity (for details and

references see Benson and Ebels, 1978, 1981; Ebels, 1979; Ebels and Benson, 1978; Pévet, 1981c, 1983b; Reiter and Vaughan, 1977). Considering the process of synchronization of sexual activity with the seasons, although to date there is no experimental proof that pineal proteic/peptidic hormones are directly involved, the study of the pineal active proteic/peptidergic fractions appears to be of fundamental interest. Such a study, however, is a very complicated one because the vertebrate pineal is known to contain an extraordinarily large number of proteic or peptidic compounds, some of them being identified or partially characterized, others not (for a detailed list, see Pévet, 1983b). Moreover, as was already explained in detail (Pévet, 1983b), all peptides and proteins extracted and isolated from pineal tissue, cannot be all considered as true pineal compounds. They may indeed be synthesized elsewhere but found in the pineal because present in nerve axons coming from other brain structures (class I), or because of a secondary uptake from the general circulation (class II). Only those proteic/peptidergic compounds synthesized in the pineal by specific pineal cells (class III) can be considered as true pineal hormones.

Consideration of such a classification of proteic/peptidergic pineal substances into three different classes is very important because, for example, the role of non-pineal substances (classes I and II) could be very important in the physiological process presently studied.

A. Peptides and proteins of the first class

Peptidergic nerve fibres originating from other brain structures are known to be present in the pineal (Ariëns Kappers, 1983b; Korf and Møller, this volume). It is this complex nervous connection between the central nervous system and the pineal gland which explains the presence of peptides of class I. Vasopressin, oxytocin, vasoactive intestinal peptide (VIP), etc. (for a more complete list and details, see Pévet, 1983b), indeed, are neurotransmitters or neuromediators present in such fibres. It is very probable that some of the endocrine effects attributed to pineal "peptides" as described in the literature are in fact due to the presence of such class I - peptides in the fraction isolated by the chemist. Oxytocin, for example, which is present in the pineal, will react in the milk-injection test.

Peptides of class I are neurotransmitters or neuromodulators. Their physiological role should thus concern the pineal itself. They probably influence pineal synthetic activity and thus transmit information coming from the brain to the pineal. Data on this aspect of the pineal peptide problem are as yet very scarce, But Kaneko *et al.* (1980) and in particular Yuwiler (1983a,b) have clearly demonstrated in organ culture that VIP, one of the relevant peptides of class I, influences the indole derivative biosynthetic pathway. VIP indeed stimulates basal serotonin N-acetyltransferase activity, its effect being apparently mediated by a receptor.

B. Peptides and proteins of the second class

As suggested by Ariëns Kappers (1978) and Cardinali (1981), the function of the pineal gland, like that of other endocrine organs, may depend on feedback systems. This peculiar aspect of pineal physiology is important to consider here. A feedback system operating between the hypothalamus or the hypophysis and the pineal would imply that the pineal accumulates circulating hypothalamic or hypophysial hormones. This aspect of the pineal peptide/protein problems has not yet been thoroughly investigated. Clear results, however, have been obtained with some hypothalamic hormones. After intravenous injection of labelled MIF (α-MSH release inhibiting factor) Redding *et al.* (1973) have observed that the radioactive hormone was especially concentrated in the pituitary and pineal gland. A similar observation was made after injection of labelled luteinizing hormone-releasing hormone (LHRH) by Redding and Schally (1973), Admundson and Wheatons (1979) and Trentini *et al.* (1980). The last-mentioned group noted also that the pineal gland accumulates ^{3}H-LHRH in an amount significantly higher than the pituitary gland. These experiments which demonstrated that the pineal is able to concentrate in a notable amount circulating MIF and LHRH, can easily explain the presence in the pineal of these hypothalamic hormones as described in the literature. Such phenomenon of accumulation may also explain the presence of other hormones such as α-MSH, TRH, ACTH, luteinizing hormone, prolactin, etc. (see details and references in Pévet, 1983b).

Again, it is quite conceivable that some endocrine effects attributed to pineal "peptides" as described in the literature, are in fact caused by the presence of such peptides or proteins of class II in the fractions isolated in the laboratory. The physiological significance of such phenomenon of accumulating circulating hormones is not yet completely known, but it concerns the pineal itself. These circulating hormones accumulated in the pineal (peptides/proteins of class II) probably influence pineal activities and so transmit information on the state of activity of the endocrine system to the pineal. Data on this aspect of the pineal peptide problem are still very scarce, but the few results obtained sofar seem to suggest that these hormones act on the biosynthetic pathway of the indole-derivatives. Cardinali *et al.* (1976) and Cardinali (1981), for example, have observed an increase in melatonin synthesis after prolactin administration and noted the presence of a putative prolactin receptor in the pineal (a prerequisite for a direct effect of prolactin on the gland).

C. Peptides and proteins of the third class

Only those proteic/peptidic compounds which are synthesized in the gland itself by specific pineal cells can be considered as true pineal factors. To date none of these substances have been structurally identified, and by consequence studying them is difficult because such studies are always indirect ones (see details in Pévet, 1981c, 1982, 1983b).

Processes involved in the synthesis and release of proteic/peptidic substances have been identified at the ultrastructural level. One is characterized by the formation of granular vesicles (GV) by the Golgi apparatus, and the other by the formation of vacuoles containing flocculent material of moderate electron density by the cisterns of the rough endoplasmic reticulum (Pévet, 1979, 1981a, 1982, 1983a,b; Collin, 1979; Collin and Oksche, 1981). Cytochemical studies have permitted to clearly demonstrate the proteinaceous nature of the material thus formed (Collin and Meiniel, 1972; Juillard, 1975; Pévet, 1977). Moreover, autoradiographical, histochemical, ultracytochemical and pharmacological investigations have shown in some species (parakeet, lizard, mouse, hamster) that serotonin coexists with a proteinaceous compound, at least in the GV (see details in Collin, 1979; Collin and Oksche, 1981).

The existence of proteins or peptides of class III cannot be discarded, but their exact role remains to be determined. These substances may indeed be *(1)* carrier proteins for serotonin or other indole derivatives, or *(2)* specific pineal hormones.

Although it cannot be completely excluded, the first possibility is not seriously considered to date. In numerous cells which produce a polypeptidergic hormone such as calcitonin by the parathyroid cells, there is a concomitant presence of the hormone and of serotonin in the granular vesicles, while a functional interrelationship has been shown to exist between the two. It is generally thought that also in the pinealocytes (or homologous cells in lower vertebrate pineals) serotonin is linked with a proteinaceous hormone, both compounds being released together into the circulation, or that serotonin may be implicated in some stage of the process of synthesis, storage, or release of the proteinaceous hormone. This recalls the situation in APUD cells or paraneurons, and pinealocytes are considered to belong to these families (Matthews and Leong, 1981; Ralph, 1983; Ueck and Wake, 1979).

The concept that the proteic/peptidic substances formed are hormones, is also strongly supported by the numerous cytophysiological experiments performed *in vivo* and *in vitro* in laboratory animals as well as in wild species. These studies have indeed allowed to demonstrate that the activity of the secretory process is regulated by circulating sex hormones, thus demonstrating the existence of a feedback system between the pineal and the gonadal axis (Pévet, 1979, 1981a,c, 1982, 1983a,b; Karasek and Reiter, 1982; Karasek, 1979, 1983; Haldar-Misra and Pévet, 1983c,d,e). Moreover, it has been demonstrated that these processes of protein secretion are also regulated by the sympathic innervation and by the various indole-derivatives, melatonin included (Benson and Krasovich, 1977; Haldar-Misra and Pévet, 1983a,b). These last-mentioned results support the old concept of Quay (1974) that the pineal itself is a target organ for melatonin.

These ultrastructural analyses, however, give only indirect evidence of the effects by melatonin or other 5-methoxyindoles on the pineal secretion of proteic/peptidic hormones. At present we have no experimental proof of such phenomenon, which is even contested by many pinealogists. Some experiments, however, oblige — in our opinion — to at least consider this possibility.

When injected late in the afternoon into male adult hamsters kept under long photoperiod, melatonin is able to induce gonadal atrophy only if the pineal is present or its innervation intact (Reiter *et al.*, 1976). This pineal-dependent effect of melatonin has been interpreted in different ways. It was assumed, for example, that *(1)* exogenous melatonin would be additive to, or would interfere with the action of endogenous melatonin, or *(2)* exogenous melatonin would stimulate or inhibit the synthesis and/or release of a true pineal "antigonadal" hormone.

Since it was demonstrated that melatonin when injected thrice a day in pinealectomized hamsters, induced gonadal quiescence (Tamarkin *et al.*, 1977), some authors have argued that an intact pineal would not be required for the manifestation of the antigonadotrophic effects of melatonin, and most of them have now abandonned the second interpretation (Tamarkin *et al.*, 1977; Reiter, 1980; Hoffmann, 1981). As indicated by Blask (1981) however, "the interpretation of their data is baffling, since much higher doses of melatonin are required to induce, in pinealectomized or denervated animals, the same response that occurs in intact hamsters receiving only one injection". Moreover, recently Pévet *et al.* (1983) have observed that under the same experimental conditions melatonin was able to induce gonadal atrophy when the pineal synthesis of serotonin (and, thus, also of other serotonin derivatives) was inhibited by benserazide, an aromatic amino-acid decarboxylase inhibitor. It is therefore possible to suggest that melatonin does not act on the gonadal axis, neither via the increased production by the pineal of serotonin derivatives, nor via a synergic effect with endogenous serotonin derivatives. Although there is no proof it is — in our opinion — probable that the pineal compound implicated is a proteic/peptidic hormone.

III. INDOLE-DERIVATIVES: THEIR ROLE

In 1917 McCord and Allen reported that extracts of bovine pineals produced a dramatic blanching of the skin in amphibians Forty-two years later, Lerner *et al.* (1959) isolated and characterized the compound responsible for this effect: N-acetyl-5-methoxytryptamine. Since this compound caused aggregation of melanin granules within melanophores, they called it melatonin. The enzymatic pathway which produces melatonin, and its dependence of the environmental lighting conditions, were then demonstrated (Axelrod, 1983; Klein *et al.*, 1981). Since that time an extraordinarily large amount of research has been performed on melatonin and its role, and to date this compound is considered as the pineal hormone responsible for inhibiting the reproductive system (details and refs. in Reiter, 1980), or as the pineal factor conveying photoperiodic messages. In short, the circadian pattern of pineal melatonin secretion is regulated by the nervous system. Changes in photoperiod, acting via the nervous system, alter the temporal pattern of melatonin secretion. It would be this change in secretion pattern which would

convey information about photoperiod to the reproductive axis and probably to other systems (for details and references see Goldman, 1983; Goldman and Darrow, 1983; Hoffmann, 1981; Hoffmann, this volume; Vivien-Roels and Pévet, 1983).

Melatonin is thus a crucial pineal compound in the presently examined physiological process, and for more details we refer to the abovementioned authors. However, the problem is even more complicated. Many other indole derivatives are also synthesized by the pineal in a rhythmic fashion, and many other organs are able to synthesize these compounds, melatonin included.

(a) *Indole derivatives different from melatonin*

It is well-known that within the pineal cells part of the amino acid tryptophan, taken up from the blood, is converted to 5-hydroxytryptophan by hydroxylation. Decarboxylation of this indole derivative leads to the formation of 5-hydroxytryptamine (serotonin) which is the indole that attains the largest concentration within the pineal tissue. Serotonin, then, can be acetylated to N-acetylserotonin, oxidized to 5-hydroxyindole-3-acetic acid, or metabolized to 5-hydroxytryptophol. The 5-hydroxyindoles, 5-hydroxytryptophan, 5-hydroxytryptamine (serotonin), 5-hydroxyindole-3-acetic acid (N-acetylserotonin) can be methylated by hydroxyindole-O-methyltransferase (HIOMT). This means that at least 5 different methylated products: 5-methoxytryptophan, 5-methoxytryptamine, 5-methoxyindole-3-acetic acid, 5-methoxytryptophol and melatonin can be formed in the pineal (Balemans, 1979, 1981). All these 5-methoxyindoles (and 5-hydroxyindoles) are potentially active factors and indeed numerous results in the literature report an effect of some of these indoles on sexual development and reproduction. More specially it has been demonstrated that 5-methoxytryptamine, like melatonin, if administered properly, can duplicate most of the effects of the photoperiod on the reproductive axis of the male golden hamster, and that the effects of 5-methoxytryptophol, like those of melatonin and 5-methoxytryptamine, are photoperiod-dependent (see details and references in Pévet, 1983c,d).

Comparing the results with those obtained by melatonin, an analysis of the secretory pattern of these 5-methoxyindoles (and hydroxyindoles) appears to be important. Although the results of this study are still scarce, clear information is available at present. Most of the 5-hydroxy- and 5-methoxyindoles appear to be synthesized in a rhythmic fashion. This was known for a long time for serotonin, the precursor of most 5-methoxyindoles of which the synthesis in the pineal is light-dependent. Concerning 5-methoxytryptophol, Pévet *et al.* (1980), using the technique of Balemans *et al.* (1978), have observed that in male hamsters there is a diurnal change in the synthesis of 5-methoxytryptophol, with a peak during daytime, a result which has recently been confirmed by Beck and Pévet (1984) (Fig. 1), using the gas chromatography-mass spectrometry technique. Much additional information, often fragmentary, has been reported on this problem of the diurnal rhythm in 5-hydroxy- and 5-methoxyindole synthesis. Comparing the results obtained in different species (reptiles, birds and mammals) it appears that the

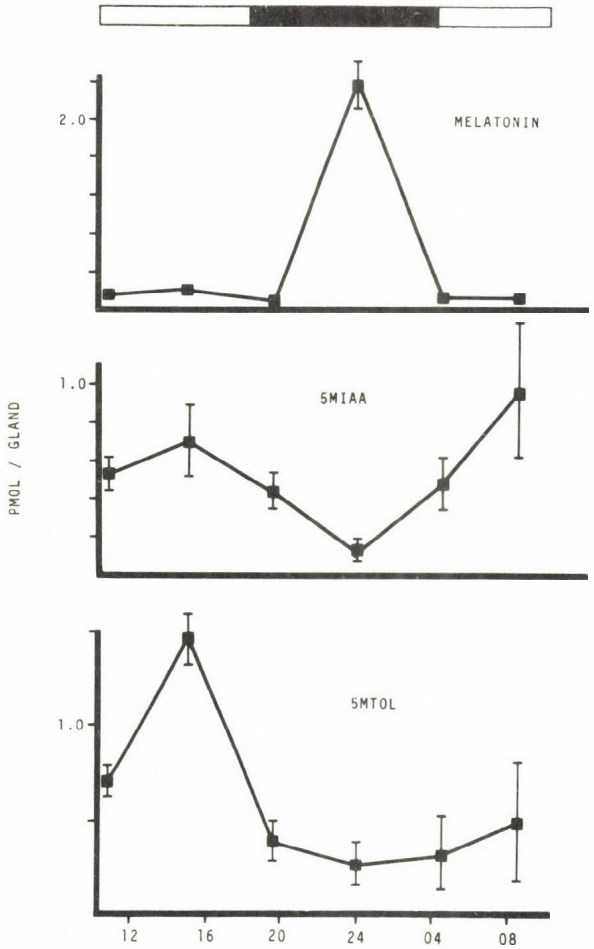

FIG. 1. Concentrations of melatonin, 5-methoxyindole-acetic acid (5MIAA) and 5-methoxytryptophol (5MTOL) in the hamster pineal gland during a light-dark cycle (long photoperiod, 14L/10D). (From Beck and Pévet, 1984, with permission).

relative importance of the various 5-methoxyindoles synthesized by the pineal, as well as the diurnal and seasonal rhythms, vary considerably from one species to another, suggesting that not only melatonin but also other 5-methoxyindoles are of physiological importance. (For a detailed analysis and references we refer to Vivien-Roels and Pévet, 1983; and Vivien-Roels, this volume.)

(b) *The extra-pineal sources of melatonin and of other 5-methoxyindoles*

Until recently it was commonly assumed that the pineal gland was the only source of melatonin. This assumption was based on early studies by Axelrod and Weissbach (1961) and Axelrod *et al.* (1961) who reported that HIOMT, the enzyme implicated in the formation of melatonin, was found only in the pineal. Since that time, however, considerable evidence has accumulated on the presence of HIOMT and of melatonin in many organs such as the Harderian gland, the retina, the intestine (Gern and Karn, 1983; Vivien-Roels and Pévet, 1983; Pévet, 1983c). Melatonin synthesis itself has been demonstrated to occur in the retina of the trout (Gern *et al.*, 1978; Gern and Ralph, 1979), the tortoise (Vivien-Roels, personal communication), mole-rat (Balemans *et al.*, 1980), hamster (Pévet *et al.*, 1980; Balemans *et al.*, 1983) and mole (Pévet *et al.*, 1978), and in the Harderian gland of the hamster (Pévet *et al.*, 1980; Balemans *et al.*, 1983) and mole-rat (Balemans *et al.*, 1980). Such extra-pineal synthesis of melatonin has also been indirectly confirmed in the rat and hamster by Reiter *et al.* (1982, 1983). These authors, indeed, observed an increase in melatonin concentration in the retina and Harderian gland after pinealectomy. Recently it has also been demonstrated that, like the pineal, the retina and the Harderian gland synthesize also other 5-methoxyindoles such as 5-methoxytryptophol, 5-methoxytryptamine, etc. (Pévet *et al.*, 1981; Balemans *et al.*, 1983; Pévet, 1983c,d).

Quantitatively this production of melatonin and other 5-methoxyindoles is not neglectable. Indeed, if in Richardson's ground squirrel (Reiter *et al.*, 1981) or the rat (Reiter *et al.*, 1983) the pineal melatonin concentration measured by RIA is far higher than that observed in the retina and Harderian gland, in some other species such as the Syrian hamster a completely opposite result was observed. Reiter *et al.*, for example (1982), found in the Harderian gland during day-time approximately 25 pg of melatonin/mg wet tissue. Considering that one hamster Harderian gland weighs 80 - 100 mg, and that one animal has two Harderian glands, this means that during day-time 4000 - 5000 pg of melatonin are present in the Harderian glands, and this should be compared with the approximately 250 pg of melatonin found in the pineal in day-time and even with the 2000 - 2500 pg found during the night. This result — which confirmed the observations by Pévet *et al.* (1980) who, using different techniques, obtained similar results — indicates clearly (especially if we consider also the retina, intestine and other as yet unidentified production organs) that only a small part of the melatonin and other 5-methoxyindoles present in the body originates from the pineal (Pévet *et al.*, 1981; Pévet, 1983c).

The physiological role of the extra-pineal melatonin and other 5-methoxyindoles is still unknown. With respect to the presently examined phenomenon of seasonal reproduction it appears that, like for the pineal, the diurnal melatonin synthesis or concentration in the retina and the Harderian gland is different, depending upon the species, the lighting conditions and the season. The very few results obtained sofar seem to demonstrate that the same holds for the other 5-methoxyindoles, and that in the retina and Harderian gland the synthesis of

5-methoxyindoles appears to be related to the light-dark cycle and other factors such as environmental temperature (Vivien-Roels and Pévet, 1983; Gern and Karn, 1983).

The 5-methoxyindoles produced by the retina and Harderian gland as well as those produced by other organs are therefore probably implicated in the phenomenon studied. The question is: how? It would seem that a local action has to be envisaged. Melatonin, for example, is known to play a role in the pigment aggregation in the epithelium of the retina (Pang and Yew, 1979) and, similarly, it may act as a substance inhibiting or facilitating the visual process by modifying the porphyrin content in the Harderian gland (Wetterberg *et al.*, 1970).

Without any doubt the local effect is important to consider. However, the question of whether part of the circulating melatonin (as well as other 5-methoxyindoles) originates from these organs is of primordial importance.

(c) *The origin of circulating 5-methoxyindoles*

To date, melatonin, 5-methoxytryptophol and 5-methoxytryptamine have been identified in blood (references in Pévet, 1983c), but the available information on the problem of their synthesis concerns exclusively melatonin. A daily rhythm in circulating melatonin has been reported for several mammals, birds, reptiles, amphibians and fishes. Generally, changes in blood melatonin show a strong parallel with changes in pineal melatonin content, and pinealectomy induces a drastic decrease in plasmatic melatonin concentration (Vivien-Roels and Pévet, 1983). However, in the tortoise *(Testudo hermanni)*, depending upon the season, such parallelism between pineal and blood melatonin concentration is not always evident. In June and August, a night peak is observed in both pineal and plasma, but in September the circulating melatonin levels remain lower during the night while a day-night rhythm is still observed in the pineal. Also, in December there is no correlation between pineal and circulating melatonin levels (Vivien-Roels and Arendt, 1981). Circulating melatonin was also observed in pinealectomized rats (Ozaki and Lynch, 1976). Kennaway *et al.* (1977) even found an increased level in sheep after pinealectomy, a result which was contested by Arendt *et al.* (1979). In trout, Gern *et al.* (1978) demonstrated that if pinealectomy significantly reduced plasma melatonin, a nycthemeral rhythm with high values in mid scotophase persisted. In the green sea turtle, pinealectomy does not either reduce nocturnal plasmatic melatonin concentration (Owens *et al.*, 1980).

The presence of melatonin has also been demonstrated in the blood of animals such as the alligator and the armadillo in which the pineal is lacking (Ralph, 1981; Roth *et al.*, 1980). In the armadillo, even a clear day-night variation with high levels during the night was detected (Harlow *et al.*, 1981). So, if in most animals the major part of the melatonin present in the blood originates from the pineal it is evident that, depending upon the species, a larger or smaller fraction of it may originate from extra-pineal sources. Such evidence is important to consider here especially because functional relation-

ships are known to exist between the pineal, the retina and the Harderian gland. Harderianectomy, for example, abolishes the effect of light on pineal HIOMT and on serotonin levels in blinded rats (Wetterberg *et al.*, 1970). Pinealectomy will also induce an increase in melatonin content in the retina and Harderian gland of the rat (Yu *et al.*, 1981; Reiter *et al.*, 1983). "Something of pineal origin may normally curtail melatonin levels within the Harderian gland (and the retina)", thus concluded the last-mentioned group. As it is known that exogenously administered 5-methoxyindoles, at least melatonin, are taken up primarily by the 5-methoxyindole-synthesizing organs themselves (pineal, Harderian gland, retina, etc.) (Wurtman *et al.*, 1964; Bubenik *et al.*, 1978), it may be suggested that the "something" could be a 5-methoxyindole. Circulating 5-methoxyindoles originating from one organ could influence the synthesis of other 5-methoxyindoles in another organ.

IV. 5-METHOXYINDOLES, 5-METHOXYINDOLE-SYNTHESIZING ORGANS, PINEAL PROTEIC/PEPTIDIC HORMONES, AND SEASONAL REPRODUCTION: A WORKING HYPOTHESIS

In the pineal gland neuronal information derived from the external light/dark cycle is transduced into a chemical message which acts at a distinct target site (Gern and Karn, 1983; Goldman, 1983). Although this phenomenon is an integral part of this process, the mechanism by which the seasonal sexual cycle is synchronized with annual climatic changes appears to be more complicated.

The numerous — often contradictory — data obtained on this problem in the last few years, in mammalian as well as in non-mammalian species, are difficult to integrate. However, it appears that *(1)* the organs which react to environmental information such as light (pineal, retina, Harderian gland), temperature (pineal, retina (?), Harderian gland), or food (intestine), synthesize 5-methoxyindoles and also concentrate circulating 5-methoxyindoles, at least melatonin; *(2)* the rhythm in melatonin concentration and synthesis, and the rhythm in the synthesis of other 5-methoxyindoles in the pineal, the retina and Harderian gland are, depending of the species and of the experimental conditions, photoperiod- as well as temperature-dependent; *(3)* under both natural and laboratory conditions, all these rhythms show different seasonal variations; *(4)* the 5-methoxyindoles strongly influence the synthesis of proteic/peptidic compounds in the pineal itself.

In order to integrate all this information, the following hypothesis has recently been proposed (Pévet *et al.*, 1981; Pévet, 1983d): "The 5-methoxyindoles (melatonin, 5-methoxytryptamine, 5-methoxytryptophol, etc.), synthesized by the pineal as well as by the retina, Harderian gland, intestine, etc.),

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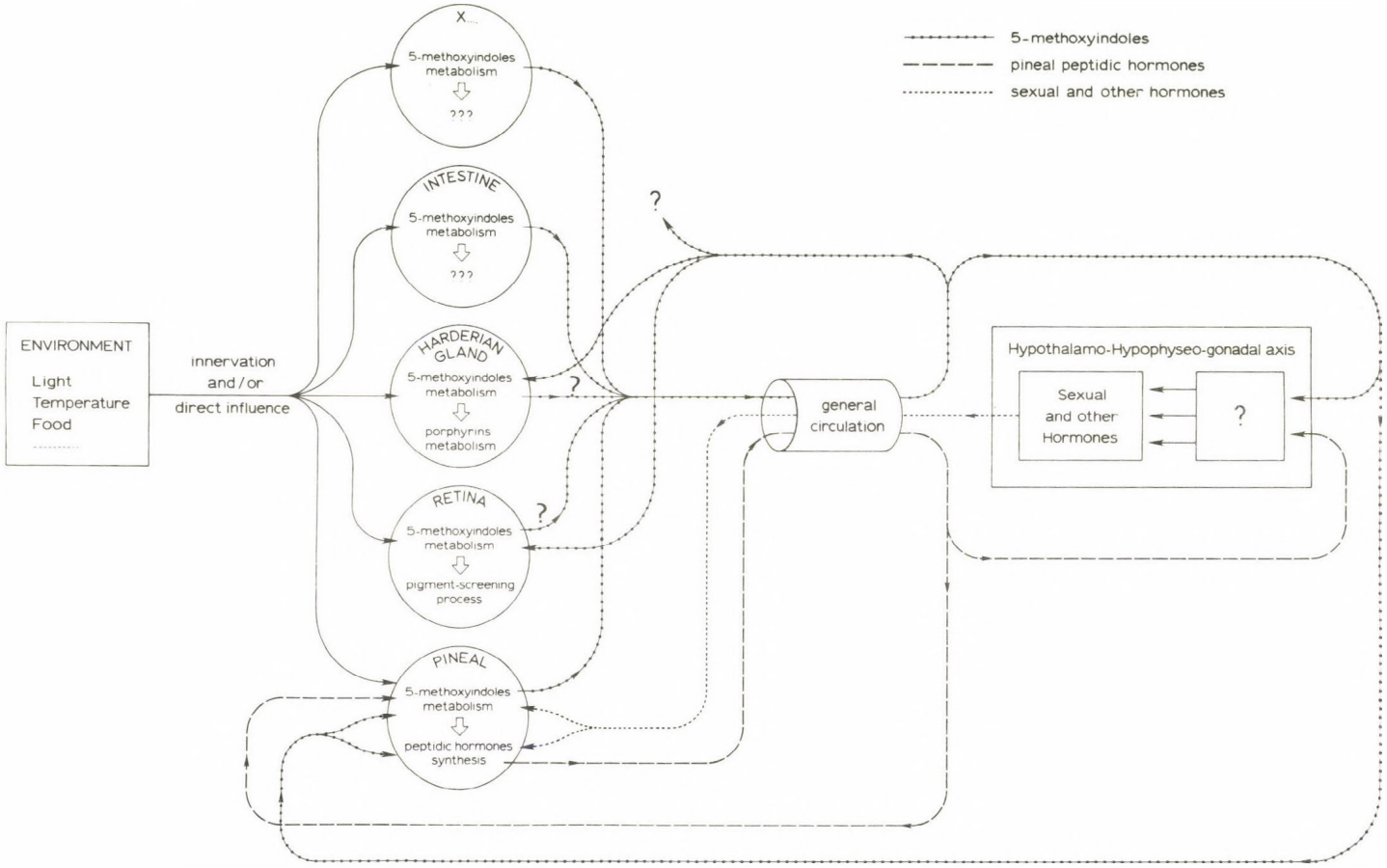
FIG. 2. Schematic summary of the working hypothesis indicated in the text.


5-methoxyindoles
pineal peptidic hormones
sexual and other hormones
X...
5-methoxyindoles metabolism
???
INTESTINE
5-methoxyindoles metabolism
???
HARDERIAN GLAND
5-methoxyindoles metabolism
porphyrins metabolism
RETINA
5-methoxyindoles metabolism
pigment-screening process
PINEAL
5-methoxyindoles metabolism
peptidic hormones synthesis
ENVIRONMENT
Light
Temperature
Food
innervation and/or direct influence
general circulation
Hypothalamo-Hypophyseo-gonadal axis
Sexual and other Hormones
?

may be implicated in a system by which the pineal and some other brain structures may be able to perceive, to differentiate and to integrate information from the environment such as photoperiod, temperature, availability of food, magnetic field, etc. In response, the pineal may synthesize and release proteic/peptidic hormone(s) which may act on the reproductive axis."

This working hypothesis, schematically presented in Figure 2, is tested at present at several levels in our laboratory. In this hypothesis, the 5-methoxyindoles play a major role. The seasonal cycle in the activity of an endocrine axis such as the reproductive axis represents a phenomenon of long-term adaptation of the brain to changes in the environment. To adapt adequately, the brain should not only perceive and differentiate environmental information, but also the state of activity of various endocrine glands. Normally, the brain is protected by the blood-brain-barrier. The pineal is one of the few brain structures outside the blood-brain-barrier. It is known that the pineal is able to concentrate circulating hormones such as prolactin, LHRH, LH, testosterone, etc. (details and references in Pévet, 1982, 1983b; Cardinali, 1981, 1983). The exact physiological significance of such phenomenon of accumulation of circulating hormone is not exactly known, but the very few results obtained so far clearly indicate that these hormones influence the synthesis of melatonin (Cardinali, 1981; Cardinali, 1983), a compound which, like 5-methoxytryptophol and 5-methoxytryptamine, is able to cross the blood-brain-barrier. By such a system the relevant brain structures would be able to integrate different information and to react adequately.

The observation by Yuwiler (1983a,b) that VIP, a neuropeptide present in peptidergic nerve fibres found in the pineal, influences also melatonin synthesis, suggests that other information coming from the brain itself could thus, at the same time, be integrated.

The circles in the diagram of Figure 2 represent the 5-methoxyindole-synthesizing organs identified to-date. A circle X has been added to indicate that in the future other such organs will probably be identified. But which organs could be the ones concerned ?

According to the present hypothesis it is via the 5-methoxyindoles that the brain integrates — for an adaptive process — environmental information. All organs which react directly or, like the mammalian pineal, indirectly via innervation to different environmental information such as e.g. the olfactory and gustatory organs and the organ of Corti, are, in our opinion, all potential 5-methoxyindole-synthesizing organs. In this respect it is interesting to note that Bubenik (1981) has identified immunocytochemically melatonin in the rat palatin gland (relation to food) and that preliminary immunocytochemical results obtained by Vivien-Roels *et al.* (personal communication) and by Vollrath (personal communication) suggest that the sensory cells of the olfactory system and of the organ of Corti of the rat contain also melatonin.

Many additional results will be needed to confirm this aspect of our hypothesis and it should be noted that recently Vivien-Roels *et al.* (1984) have demonstrated the presence of melatonin in the compound eye of an insect, the locust.

The local effect of 5-methoxyindoles in the organs in which they are synthesized are represented here because we believe that, although difficult to analyze (see details in Collin (1979) and Collin and Oksche, 1981), this problem merits consideration. Recent studies by Haldar-Misra and Pévet (1983a,b) have clearly demonstrated that melatonin and other 5-methoxyindoles have a profound influence on the activity of the process involved in the synthesis and release of proteic/peptidic compounds. Such working hypothesis raises many questions, two of which are of special interest:

- Are the different 5-methoxyindoles involved in the integration of *all* environmental information, or does a specialization of some compounds for a given information exist?
- Which is the exact role of the various 5-methoxyindole-synthesizing organs?

Concerning the first question an analysis of the problem has already been given (Vivien-Roels and Pévet, 1983; Vivien-Roels, 1983, and this volume). In short, it appears that the relative importance of the different indole-derivatives in mediating the effect of a given environmental factor may vary from one class of vertebrates to another, and even from species to species. Results obtained in reptiles, e.g., suggest that the 5-hydroxyindole serotonin could be more specially implicated in the integration of the photoperiod, whereas the 5-methoxyindole melatonin could be involved more particularly in the integration of environmental temperature (Vivien-Roels and Arendt, 1981). On the other hand it is also well-known that melatonin — the compound most studied — is thoroughly implicated in conveying photoperiodic information (Hoffmann, this volume), but in some species, especially poikilothermal species, melatonin is also able to integrate variations in environmental temperature (Vivien-Roels, this volume). Semm *et al.* (1982) and Welker *et al.* (1983) observed that in the rat the nocturnal peak in melatonin concentration is modified by an alteration of the magnetic field. One might think that the duration of the night rise in pineal melatonin is controlled by photoperiod, while its amplitude is controlled by other factors such as temperature or magnetic field. Considering the number of 5-methoxyindoles identified until now, many possibilities are open.

With regard to the problem of the possible role of other 5-methoxyindole-synthesizing organs the available information is still more scarce. Recently however, we have obtained results which permit to assert that the Harderian gland is important in such an adaptive process. Adult male golden hamsters kept under natural conditions were blinded at the end of April and sacrificed two months later. The classically described gonadal atrophy was observed, but we also found that blinding had induced atrophy of the Harderian gland, as appeared from a decrease in volume and weight. Moreover, a concomitant decrease in serotonin concentration was noted (Table 1).

The mole-rat (*Spalax ehrenbergi*, Nehring) is a blind fossorial animal in which the visual pathway is not functional (Haim *et al.*, 1983), but that responds to photoperiodic changes, as is evident from photoperiodic changes in their thermoregulatory capacities. These animals adaptively increase their resistance to cold when kept under short photoperiod (Haim *et al.*, 1983). After two weeks in short photoperiod the thermoregulatory capac-

TABLE 1. Effect of blinding on the Harderian gland of male golden hamsters kept under natural conditions. The animals were blinded at the end of April and sacrificed two months later (Pévet *et al.*, unpublished data)

	Weight mg	Serotonin content ng/mg Harderian gland	Serotonin content ng/gland
Normal hamsters (40)	89.41 ± 1.62	8.83 ± 0.15	770.53 ± 17.7
Blinded hamsters (48)	45.31 ± 3.88	5.8 ± 0.37	254.67 ± 27.08

ities of Harderianectomized animals are less than those of the controls (Pévet *et al.*, 1984. In this animal the Harderian gland is clearly implicated in the detection of photoperiodic changes.

Such a working hypothesis would also permit to explain the many other functions attributed to the pineal gland (see Relkin, 1983). 5-Methoxyindoles synthesized by, among other organs, the pineal would be involved in a system enabling the brain to integrate environmental changes. If this is true, it is evident that such a system could be used by all centrally controlled physiological systems which have to adapt to annual climate changes, the seasonal sexual cycle being only one of them. All experiments at the level of the pineal would have an effect simply because so the mechanism to integrate environmental changes is disturbed and the physiological process concerned cannot adapt to these changes anymore.

V. CONCLUSION

This working hypothesis — which is an oversimplification — probably raises more questions than it solves. All data obtained to date on different groups of vertebrates indicate that the function of melatonin and other 5-methoxyindoles is primarily concerned with the synchronization of central functions with changes in the environmental conditions. 5-Methoxyindoles are synthesized by different organs, many of which are probably still to be discovered, and this fact should not be neglected.

The environmental stimuli appear to be more specially transmitted via changes in the plasmatic concentration of different 5-methoxyindoles. In this respect, especially if we consider melatonin and photoperiodism, the pineal seems to play a central role because a larger or smaller fraction of circulating melatonin, depending upon the species, originates from the pineal.

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REFERENCES

Amundson, B.C. and Wheaton, J.E. (1979) Effects of chronic LHRH treatment on brain LHRH content, pituitary and plasma LH and ovarian follicular activity in the anestrous ewe. Biol. Reproduct. *20*, 633-638.

Arendt, J., Forbes, J.M. and Brown, N.B. (1979) Effect of pinealectomy on immunoassayable melatonin in sheep. Proc. Soc. Endocrinol. *11*, 22-23.

Ariëns Kappers, J. (1978) Localization of indoleamine and protein synthesis in the mammalian pineal gland. J. Neural Transm., suppl. *13*, 13-24.

Ariëns Kappers, J. (1983) Innervation of the vertebrate pineal organ. In *The Pineal Gland and its Endocrine Role*, J.Axelrod, F.Fraschini and G.P.Velo (eds.), Plenum Press, New York, pp. 87-112.

Axelrod, J. (1983) Regulation or circadian rhythms of indoleamines in pineal gland. In *The Pineal Gland and its Endocrine Role*, J.Axelrod, F.Fraschini and G.P.Velo (eds.), Plenum Press, New York, pp. 1-14.

Axelrod, J., MacLean, P.D., Albers, R.W. and Weissbach, H. (1961) Regional distribution of methyl transferase enzymes in the nervous system and glandular tissues. In *Regional Neurochemistry*, S.S.Kety and J.Elkes (eds.), Pergamon Press, New York, p. 301.

Axelrod, J., and Weissbach, H. (1961) Purification and properties of hydroxyindole-O-methyltransferase. J. Biol. Chem. *236*, 211-213.

Balemans, M.G.M. (1979) Indole metabolism in the pineal gland of the rat: some regulatory aspects. In *The Pineal Gland of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res. vol. 52, Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 221-229.

Balemans, M.G.M. (1981) Indole metabolism in the pineal gland, the Harderian gland and the retina of mammals. In *The Pineal Gland: Photobiology – Biochronometry – Endocrinology*, A.Oksche and P.Pévet (eds.), Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 261-280.

Balemans, M.G.M., Noordegraaf, E.M., Bary, F.A.M. and Van Berlo, M.F. (1978) Estimation of methylating capacity of the pineal gland with special reference to indole metabolism. Experientia *34*, 887-888.

Balemans, M.G.M., Pévet, P., Legerstee, W.C. and Nevo, E. (1980) Preliminary investigations on melatonin and 5-methoxytryptophol synthesis in the pineal, retina and Harderian gland of the mole rat and in the pineal of the mouse "eyeless". J. Neural Transm. *49*, 247-255.

Balemans, M.G.M., Pévet, P., Van Benthem, J., Haldar-Misra, C.,

Smith, I. and Hendriks, H. (1983) Day/night rhythmicity in the methylating capacities for different 5-hydroxyindoles in the pineal, the retina and the Harderian gland of the Golden hamster *(Mesocricetus auratus)* during the annual seasons. J. Neural Transm. *56*, 53-72.

Beck, O. and Pévet, P. (1984) Analysis of melatonin, 5-methoxytryptophol and 5-methoxyindoleacetic acid in the pineal gland and retina of hamster by capillary column-gas chromatography-mass spectrometry. J. Chromatogr. Biomed. Appl. (in press).

Benson, B. and Ebels, I. (1978) Pineal peptides. J. Neural Transm. suppl. *13*, pp. 157-174.

Benson, B. and Ebels, I. (1981) Other pineal peptides and related substances – Physiological implications for reproductive biology. In *The Pineal Gland, Vol. II, Reproductive Effects*, CRC Press, Boca Raton, Florida, USA, pp. 165-187.

Benson, B. and Krasovich, M. (1977) Circadian rhythm in the number of granulated vesicles in the pinealocytes of mice. Effect of sympathectomy and melatonin treatment. Cell Tiss. Res. *184*, 499-506.

Blask, D.E. (1981) Potential sites of action of pineal hormones within the neuroendocrine-reproductive axis. In *The Pineal Gland, Vol. II, Reproductive Effects*, CRC Press, Boca Raton, Florida, USA, pp. 189-216.

Bubenik, G.A. (1981) Immunological localization of melatonin in the salivary glands of the rat. In *Melatonin – Current Status and Perspectives*, N.Birau and W.Schloot (eds.), Pergamon Press, Oxford, pp. 391-395.

Bubenik, G.A., Purtill, R.A., Brown, G.M. and Grota, L.J. (1978) Melatonin in the retina and the Harderian gland. Ontogeny, diurnal variations and melatonin treatment. Exp. Eye Res. *27*, 323-334.

Cardinali, D.P. (1981) Melatonin. A mammalian pineal hormone. Endocr. Rev. *2*, 327-346.

Cardinali, D.P. (1983) Molecular mechanisms of neuroendocrine integration in the central nervous system: an approach through the study of the pineal gland and its innervating sympathetic pathway. Psychoneuroendocrinology *8*, 3-30.

Cardinali, D.P., Nagle, C.A. and Rosner, J.M. (1976) Gonadotrophin and prolactin induced increase of rat pineal hydroxyindole-O-methyltransferase involvement of the sympathetic nervous system. J. Endocrinology *68*, 341-342.

Collin, J.-P. (1979) Recent advances in pineal cytochemistry. Evidence of the production of indoleamines and proteinaceous substances by rudimentary photoreceptor cells and pinealocytes of amniota. In *The Pineal Gland of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res., vol. 52, Elsevier, Amsterdam, pp. 271-296.

Collin, J.-P. and Meiniel, A. (1972) L'organe pinéal du genre *Lacerta* (Reptile, lacertilien): action d'enzymes protéolytiques sur les grains denses (500-3400 Å) des photorécepteurs rudimentaires. C.R. Société Biologie *166*, 370-380.

Collin, J.-P. and Oksche, A. (1981) Structural and functional relationships in the mammalian pineal gland. In *The Pineal Gland, Vol. 1, Anatomy and Biochemistry*, R.J.Reiter (ed.), CRC Press, Boca Raton, Floroda, USA, pp. 27-68.

Czyba, J.C., Girod, C. and Durand, N. (1964) Sur l'antagonisme

épiphysohypophysaire et les variations saisonnières de la spermatogenèse chez le Hamster doré *(Mesocricetus auratus)*, C.R. Soc. Biol. *158*, 742-745.

DeLany, M.J. and Happolid, D.C.D. (1979) *Ecology of African Mammals*, Longman, London.

Ebels, I. (1979) A chemical study of some biologically active pineal fractions. In *The Pineal of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res., vol. 52, Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 309-321.

Ebels, I. and Benson, B. (1978) A survey of the evidence that unidentified pineal substances affect the reproductive system in mammals. In *The Pineal and Reproduction*, R.J.Reiter (ed.), S.Karger, Basel, pp. 51-89.

Gern, W.A. and Karn, C.M. (1983) Evolution of melatonin's functions and effects. In *Pineal Research Reviews, Vol. 1*, R.J.Reiter (ed.), Alan R. Liss, Inc., New York, pp. 49-91.

Gern, W.A., Owens, D.W. and Ralph, C.L. (1978) The synthesis of melatonin by the trout retina. J. Exp. Zool. *206*, 269-270.

Gern, W.A. and Ralph, C.L. (1979) Melatonin synthesis by the retina. Science *204*, 183-184.

Goldman, B.D. (1983) The physiology of melatonin in mammals. In *Pineal Research Reviews, Vol. 1*, R.J.Reiter (ed.), Alan R. Liss, Inc., New York, pp. 145-181.

Goldman, B.D. and Darron, J.W. (1983) The pineal gland and mammalian photoperiodism. Neuroendocrinology *37*, 386-396 (1983).

Haim, A., Heth, G., Pratt, H. and Nevo, E. (1983) Thermoregulatory responses to changes in photoperiod in the "blind" mole-rat *Spalax ehrenbergi*. J. Exp. Biol. *107*, 59-64.

Haldar-Misra, C. and Pévet, P. (1983a) The influence of different 5-methoxyindoles on the process of protein/peptide secretion characterized by the formation of granular vesicles in the mouse pineal gland. An *in vitro* study. Cell Tiss. Res. *230*, 113-126.

Haldar-Misra, C. and Pévet, P. (1983b) Influence of melatonin on the process of protein and/or peptide secretion in the pineal gland of the rat and hamster: An *in vitro* study. Cell Tiss. Res. *231*, 78-83.

Haldar-Misra, C. and Pévet, P. (1983c) The influence of luteinizing hormone releasing hormone on the process of protein and/or peptide secretion characterized by the formation of granular vesicles in the mammalian pinealocytes. Comparative *in vitro* study in three species of rodents. Cell Tiss. Res. *232*, 529-538.

Haldar-Misra, C. and Pévet, P. (1983d) Influence of testosterone on protein/peptide secretion as characterized by the formation of granular vesicles in rat and mouse pinealocytes. An *in vitro* study. J. Neural Transm. *57*, 111-120.

Haldar-Misra, C. and Pévet, P. (1983e) Influence of prolactin on the processes of protein and/or peptide secretion in mouse and rat pinealocytes. J. Neural Transm. *58*, 245-259.

Harlow, H.J., Phillips, J.A. and Ralph, C.L. (1981) Day-night rhythm in plasma melatonin in a mammal lacking a distinct pineal gland, the nine-banded armadillo. Gen. Comp. Endocrinol. *45*, 212-218.

Hoffmann, K. (1981) Photoperiodic function of the mammalian pineal organ. In *The Pineal Organ: Photobiology – Biochronom-*

etry — Endocrinology, A.Oksche and P.Pévet (eds.), Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 123-138.

Hoffman, R.A. and Reiter, R.J. (1965) Pineal gland influence on gonads of male hamsters. Science *142*, 1609-1611.

Juillard, M.T. (1979) The proteinaceous content and possible physiological significance of dense-cored vesicles in hamster and mouse pinealocytes. Ann. Biol. anim. Biochem. Biophys. *19*, 413-428.

Kaneko, T., Cheng, P.Y., Oka, H., Oda, T., Yanaihara, N. and Yanaihara, C. (1980) Vasoactive intestinal polypeptide stimulates adenylate cyclase and serotonin N-acetyltransferase activities in the rat pineal *in vitro*. Biochem. Res. *1*, 84-87.

Karasek, M. (1979) Ultrastructural study of the pineal-adenohypophyseal relationships in rats. In *The Pineal Gland of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res., vol. 52, Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 195-199.

Karasek, M. (1983) Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. In *Pineal Research Reviews, Vol. 1*, R.J.Reiter (ed.), Alan R.Liss, Inc., New York, pp. 2-48.

Karasek, M. and Reiter, R.J. (1982) A reciprocal relationship between the adenohypophysis and the pineal gland. Medical Hypothesis *9*, 1-9.

Kennaway, D.J., Frith, R.G., Phillipou, G., Matthews, C.D. and Seamark, R.F. (1977) Specific radioimmunoassay in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. Endocrinology *101*, 119-127.

Klein, D.C. (1982) *Melatonin Rhythm Generating System*, S.Karger, Basel.

Klein, D.C., Auerbach, D.A., Namboodiri, M.A.A. and Wheler, G.H.T. (1981) Indole metabolism in the mammalian pineal gland. In *The Pineal Gland, Vol. 1, Anatomy and Biochemistry*, R.J.Reiter (ed.), CRC Press, Boca Raton, Florida, USA, pp. 199-228.

Legait, H., Bauchot, R., Stephan, H. and Contet-Audonneau, J.L. (1976) Etude des corrélations liant le volume de l'épiphyse au poids somatique et encéphalique chez les rongeurs, les insectivores, les chiroptères, les prosimiens et les simiens. Mammalia *40*, 327-331.

Lerner, A.B., Case, J.D. and Heinzelman, R.V. (1959) Structure of melatonin. J. Amer. Chem. Soc. *81*, 6084.

Lofts, B. (1974) *Physiology of the Amphibians, Vol. II, Reproduction*, Academic Press, New York, USA.

Matthews, C.D. and Leong, A.Y.S. (1981) A possible role of the pineal gland and melatonin in the diffuse neuroendocrine system (DNES). In *Melatonin — Current Status and Perspectives*, N.Birau and W.Schloot (eds.), Pergamon Press, New York, pp. 77-82.

McCord, C.P. and Allen, F.P. (1917) Evidences associating pineal gland function with alterations in pigmentation. J. Exp. Zool. *23*, 207-224.

Owens, D.W., Gern, W.A. and Ralph, C.L. (1978) The effects of pinealectomy and sham-pinealectomy on serum melatonin in the green sea-turtle. Am. Zool. *18*, 624-626.

Owens, D.W., Gern, W.A. and Ralph, C.L. (1980) Melatonin in the blood and cerebrospinal fluid of the green sea turtle (*Chelonia mydas*), Gen. Comp. Endocrinol. *40*, 180-187.

Ozaki, Y. and Lynch, H.J. (1976) Presence of melatonin in plasma and urine in pinealectomized rats. Endocrinology *99*, 641-644.

Pang, S.F. and Yew, D.T. (1979) Pigment aggregation by melatonin in the retinal pigment epithelium and choroid of guinea pigs, *Cavia porcellus*. Experientia *35*, 231-233.

Perry, J.S. and Rowlands, I.W. (1972) *The Environment and Reproduction in Mammals and Birds*. Blackwell Scientific Publications, Oxford, U.K.

Pévet, P. (1977) The pineal gland of the mole (*Talpa europaea* L.). IV. Effect of pronase on material present in cisternae of the granular endoplasmic reticulum of pinealocytes. Cell Tiss. Res. *182*, 215-219.

Pévet, P. (1979) Secretory processes in the mammalian pinealocytes under natural and experimental conditions. In *The Pineal Gland of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res. 52, Elsevier, Amsterdam, pp. 149-194.

Pévet, P. (1981a) Ultrastructure of the mammalian pinealocytes. In *The Pineal, Anatomy and Biochemistry*, R.J.Reiter (ed.), CRC Press, Boca Raton (Fl.), USA, pp. 121-154.

Pévet, P. (1981b) Cytological aspects of indoleamine and protein secretion in the pineal gland. In *Human Reproduction*, K.Semm and L.Mettler (eds.), Excerpta Medica Int. Congress Series No. 551. Amsterdam, pp. 544-549.

Pévet, P. (1981c) Peptides in the pineal gland of vertebrates. Ultrastructural, histochemical, immunocytochemical and radioimmunological aspects. In *The Pineal Organ: Photobiology — Biochronometry — Endocrinology*, A.Oksche and P.Pévet (eds.), Elsevier/North-Holland Biomed. Press, Amsterdam, pp. 211-235.

Pévet, P. (1981d) Cytological aspects of indoleamine secretion in the pineal gland. In *Melatonin — Current Status and Perspectives*, N.Birau and W.Schloot (eds.), Pergamon Press, Oxford, pp. 23-33.

Pévet, P. (1982) Pineal peptides in the fetus and in young and adult mammals. In *Melatonin Rhythm Generating System*, D.C. Klein (ed.), S.Karger, Basel, pp. 155-181.

Pévet, P. (1983a) Anatomy of the mammalian pineal gland. In *The Pineal Gland*, R.Relkin (ed.), Elsevier, New York, pp. 1-75.

Pévet, P. (1983b) The different classes of proteic and peptidergic substances present in the pineal gland. In *The Pineal Gland and its Endocrine Role*, J.Axelrod, F.Fraschini and G.P.Velo (eds.), Plenum Press, New York and London, pp. 113-159.

Pévet, P. (1983c) The 5-methoxyindoles different from melatonin: Their effects on the sexual axis. In *The Pineal Gland and its Endocrine Role*, J.Axelrod, F.Fraschini and G.P.Velo (eds.), Plenum Press, New York and London, pp. 331-348.

Pévet, P. (1983d) Is 5-methoxytryptamine a pineal hormone? Psychoneuroendocrinology *8*, 61-73.

Pévet, P., Balemans, M.G.M., Legerstee, W.C. and Vivien-Roels, B. (1980) Circadian rhythmicity of the activity of hydroxyindole-O-methyltransferase (HIOMT) in the formation of melatonin and 5-methoxytryptophol in the pineal, retina and Harderian gland of the golden hamster. J. Neural Transm. *49*, 229-245.

Pévet, P., Haldar-Misra, C. and Öcal, T. (1981) The independency of an intact pineal gland of the inhibition by 5-methoxy-

tryptamine of the reproductive organs in the male hamster. J. Neural Transm. *52*, 95-106.

Pévet, P., Heth, G., Haim, A. and Nevo, E. (1984) Photoperiod perception in the blind mole-rat (*Spalax ehrenbergi*, Nehring). Involvement of the Harderian gland, atrophied eye and melatonin. J. experimental Zool. (in press).

Pévet, P. and Kuyper, M.A. (1978) The ultrastructure of pinealocytes in the golden mole *(Amblysomus hottentotus)* with special reference to the granular vesicles. Cell Tiss. Res.*191*, 39-56.

Pévet, P., Vivien-Roels, B. and Haldar-Misra, C. (1983) The gonadal atrophy induced by melatonin in the golden hamster does not implicate a pineal serotonin-dependent mechanism. Neuroendocrinology Letters *5*, 398.

Pévet, P. and Yadav, M. (1980) The pineal gland of equatorial mammals. 1) The pinealocytes of the Malaysian rat *(Rattus sabanus)*. Cell Tiss. Res. *210*, 417-433.

Quay, W.B. (1974) *Pineal Chemistry*, Charles Thomas, Springfield, Ill., USA.

Quay, W.B. (1980) Greater pineal volume at higher latitudes in Rodentia: exponential relationship and its biological interpretation. Gen. Comp. Endocrinol. *41*, 340-348.

Ralph, C.L. (1975) The pineal gland and geographical distribution of animals. Int. J. Biomet. *19*, 289-303.

Ralph, C.L. (1981) Melatonin production by extrapineal tissue. In *Melatonin — Current Status and Perspectives*, N.Birau and W.Schloot (eds.), Pergamon Press, Oxford, pp. 35-46.

Ralph, C.L. (1983) Evolution of pineal control of endocrine function in lower vertebrates. Amer. Zool. *23*, 597-605.

Redding, T.W., Kastin, A.J., Nair, R.M.G. and Schally, A.V. (1973) Distribution, half-life and excretion of ^{14}C and ^{3}H-labeled L-prolyl-L-leucyl-glycinamide in the rat. Neuroendocrinology *11*, 92.

Redding, T.W. and Schally, A.V. (1973) The distribution, half-life and excretion of tritiated luteinizing hormone-releasing hormone (LHRH) in rats. Life Science *12*, 23-32.

Reiter, R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. Endocrinol. Rev. *1*, 109-131.

Reiter, R.J., Blask, D.E., Johnson, L.Y., Rudeen, P.K., Vaughan, M.K. and Waring, P.J. (1976) Melatonin inhibition of reproduction in the male hamster: its dependency on time of day of adminitration and an intact sympathetically innervated pineal gland. Neuroendocrinology *22*, 107-116.

Reiter, R.J., Richardson, B.A. and Hurlbut, E.C. (1981) Pineal, retinal and Harderian gland melatonin in a diurnal species, the Richardson's ground squirrel *(Spermophilus richardsonii)*. Neurosci. Lett. *22*, 285-288.

Reiter, R.J., Richardson, B.A., Matthews, S.A., Lane, S.J. and Ferguson, B.N. (1983) Rhythm in immunoreactive melatonin in the retina and Harderian gland of rats: persistance after pinealectomy. Life Sciences *32*, 1229-1236.

Reiter, R.J., Trakulrungsi, W.K., Trakulrungsi, C., Vriend, J., Morgan, W.W., Vaugham, M.K., Johnson, L.Y. and Richardson, B.A. (1982) Pineal melatonin production: Endocrine and age effects. In *Melatonin Rhythm Generating System: Developmental Aspects*, D.C.Klein (ed.), Karger, Basel, pp. 143-154.

Reiter, R.J. and Vaughan, M.K. (1977) Pineal antigonadotrophic substances: polypeptides and indoles. Life Science *21*, 139-150.

Relkin, R. (1983) *The Pineal Gland*. Elsevier Scientific Publ. Co., New York, USA.

Roth, J.J., Gern, W.A., Roth, E.C., Ralph, C.L. and Jacobson, E. (1980) Non pineal metabolism in the alligator *(Alligator mississippiensis)*. Science *210*, 548-550.

Semm, P., Schneider, T., Vollrath, L. and Wiltschko, W. (1982) Magnetic sensitive pineal cells in pigeon. In *Avian Navigation*, Papi and Walraff (eds.), Springer Verlag, Berlin, pp. 329-337.

Tamarkin, L., Hollister, C.W., Lefebvre, N.G. and Goldman, B.D. (1977) Melatonin induction of gonadal quiescence in pinealectomized hamsters. Science *198*, 953-955.

Trentini, G.P., De Gaetani, C.F., Di Gregorio, C. and Botticelli, C.S. (1980) LHRH incorporation in normal and denervated pineal gland and in pineal gland of rats with constant estrous-anovulatory syndrome: a preliminary study. Endokrinologie *76*, 6-12.

Ueck, M. and Wake, K. (1979) The pinealocyte — A paraneuron. In *The Pineal Gland of Vertebrates including Man*, J.Ariëns Kappers and P.Pévet (eds.), Prog. Brain Res., vol. 52, Elsevier, Amsterdam, pp. 141-147.

Vivien-Roels, B. (1983) The pineal gland and the integration of environmental information: possible role of hydroxy- and methoxyindoles. Molec. Physiol. *4*, 331-351.

Vivien-Roels, B. and Arendt, J. (1981) Environmental control of pineal and gonadal function: Preliminary results on the relative roles of photoperiod and temperature. In *Les Colloques de l'INRA. Photoperiodism and Reproduction*, Vol. 6, pp. 273-288.

Vivien-Roels, B. and Pévet, P. (1983) The pineal gland and the synchronization of reproductive cycles with variations of the environmental climatic conditions, with special reference to temperature. In *Pineal Research Review*, R.J.Reiter (ed.), Alan R.Liss Inc., New York, pp. 92-143.

Vivien-Roels, B., Pévet, P., Beck, O. and Fèvre-Montange, M. (1984) Identification of melatonin in the compound eyes of an insect, the locust *(Locusta migratoria)* by radioimmunoassay and gas chromatography-mass spectrometry. Neurosci. Lett., vol. 49, 153-157.

Vivien-Roels, B., Pévet, P., Dubois, M.P., Arendt, J. and Brown, G.M. (1981) Immunohistochemical evidence for the presence of melatonin in the pineal gland, the retina and the Harderian gland. Cell Tiss. Res. *217*, 105-115.

Welker, H.A., Semm, P., Willig, R.P., Commentz, J.C., Wiltschko, W. and Vollrath, L. (1983) Effect of an artificial magnetic field on serotonin N-acetyltransferase. Activity and melatonin content of the rat pineal gland. Exp. Brain Res. *50*, 426-432.

Wetterberg, L., Geller, E. and Yuwilder, A. (1970) Harderian gland: an extraretinal photoreceptor influencing the pineal gland in neonatal rats. Science *167*, 884.

Wurtman, R.J., Axelrod, J. and Potter, L.T. (1964) The uptake of H^3-melatonin in endocrine and nervous tissues and the effects of constant light exposure. J. Pharmacol. exp. Ther. *143*, 314-318.

Yu, H.S., Pang, S.F. and Tang, P.L. (1981) Increase in the level of retinal melatonin and persistence of its diurnal rhythm in rats after pinealectomy. J. Endocr. *91*, 477-481.

Yuwiler, A. (1983a) Vasoactive intestinal peptide stimulation of pineal serotonin-N-acetyltransferase activity: General characteristics. J. Neurochem. *41*, 146-153.

Yuwiler, A. (1983b) Light and agonists after pineal N-acetyltransferase. Induction by vasoactive intestinal polypeptide. Science *220*, 1082-1083.

Zweig, M., Snyder, S.H. and Axelrod, J. (1966) Evidence for a nonretinal pathway of light to the pineal gland of newborn rats. Proc. Natl. Acad. Sci. U.S.A. *56*, 515-520.

The Pineal Gland
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INTERACTIONS BETWEEN PHOTOPERIOD, TEMPERATURE, PINEAL AND SEASONAL REPRODUCTION IN NON-MAMMALIAN VERTEBRATES

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Seasonal reproduction in most species appears closely correlated to climatic changes, and animals have thus developed adaptative strategies in order to synchronize their reproductive activity with the period of the year where conditions of life are optimal for the survival of the offspring.

Periodic environmental cycles, such as photoperiod, temperature or rainfall are known to give predictive information and are used by many species as indicators of seasons. If the exact mechanisms by wich environmental informations are integrated are not yet known, the pineal gland appears to be involved in long term adaptation of seasonal reproduction (ref in : Reiter 1978, 1981, Kappers and Pévet 1979, Oksche and Pévet 1981, Follett and Follet 1981, Vivien-Roels and Pévet 1983, Hoffman this volume).

The relationships existing between the development of the pineal gland and the zoogeographical distribution or the ecological conditions in which animals live, suggest that the pineal gland may be implicated in the integration of environmental information (Ralph, 1975, Pévet, 1979, this volume, Vivien-Roels, 1981, Vivien-Roels and Pévet, 1983), the gland being well-developed when the pressure of the environment is very strong. It is now also clearly demonstrated that some pineal hydroxy- and methoxy-indoles are implicated in mediating the effects of photoperiodic information on seasonal reproduction in many vertebrates living in temperate regions (ref in Goldman, 1983).

Photoperiod, however, is only one environmental factor that vary annualy and in many non-mammalian vertebrates, especially in poïkilotherms, seasonal variations in environmental temperature are known to be more important than photoperiod in the regulation of seasonal reproductive cycles (Lofts, 1975).

This raises the question whether the pineal gland, which in many poïkilotherms is well-developed and active, is able to integrate more than one environmental factor.

This review will be an attempt to specify the possible role of the pineal gland and more especially of the indole derivatives in mediating the effects of environmental information, with special reference to temperature, on seasonal reproduction in non-mammalian vertebrates.

INTERACTIONS BETWEEN PHOTOPERIOD AND TEMPERATURE IN REGULATING SEASONAL REPRODUCTIVE CYCLES IN NON-MAMMALIAN VERTEBRATES

In order to anticipate the approach of suitable season for breeding activity, animals have evolved response mechanisms to various appropriate environmental stimuli, such as photoperiod, temperature, humidity, rainfall. Among these "proximate factors", as defined by Baker (1938), photoperiod has been, by far, the most extensively studied in vertebrates as well in invertebrates (ref. in Lofts, 1975, Perry and Rowlands 1973, Follett and Follett, 1981). But although daylength is an important source of information in many seasonal cycles, it must be remembered that it is not the only factor that vary annually in mid or high latitudes. Especially, when considering poïkilothermic vertebrates, which body temperature and consequently metabolic activities change when environmental temperature varies, seasonal thermal fluctuations are of great importance (Lofts, 1975). We will thus consider more especially the interactions between these two "proximate factors" in the regulation of seasonal reproduction in non-mammalian vertebrates.

The first demonstrable proof that daylength could have an

effect on reproductive behaviour in vertebrates has been provided by Rowan (1925) in birds. For this reason, photoperiod has been extensively studied in this group (ref. in Follett and Follett, 1981). Nevertheless if it is well-known that in several birds from high and mid-latitudes the increasing daylength in spring is responsible for starting gonadal activity, it has also been reported that this gonadal response to stimulatory photoperiod may be modified by environmental temperature (Lofts, 1975). Especially some field data report correlations between environmental temperature and the speed of testicular development in response to increasing daylength, or the date of egg laying. An exceptionally mild spring for example causes unusually early breeding in many passerine birds, while a cold winter can nullify the effects of spring daylength (Lofts 1975) Nevertheless experimental data concerning interactions between photoperiod and environmental temperature on birds are lacking to date.

In poïkilotherms, the effects of daylength on reproduction are generally inextrically intermingled with those of temperature an in many species thermoperiodism is often dominant to photoperiodism.

The extensive literature on reptilian reproductive cycles (ref. in Licht, 1972, Callard and Ho, 1980) reports that temperature fluctuations greatly affect annual gonadal cycles, but often associated with photoperiod. In some species such as the lizard Anolis carolinensis, the regenerative phase of spermiogenesis is temperature dependent from late fall to early spring, but photoperiod is important in testicular maintainance and eventual regression (Callard and Ho, 1980). In the same species it has been recently demonstrated that the photoperiodic gonadal response disappears when the environmental temperature is too low (Underwood and Hall, 1982). Also in a desert lizard, Uma notata, Lofts (1975) has reported that increasing photoperiod in early spring stimulates development of the gonads while the decline of the breeding season in late spring is due to an increase of the environmental temperature in the early afternoon. In the tortoise, Testudo hermanni,

Kuchling (1982) recently reported that photoperiod had no effect on the begining of the spermatogenic cycle; a long photoperiod associated with high temperature nevertheless has a stimulatory effect on spermiogenesis. In the same species, evaluating the relative importance of photoperiod and temperature. Vivien-Roels and Arendt (1981), Vivien-Roels and Pévet (1983), also reported that the gonadal fuction of male tortoises, at least concerning testis growth and testosterone production, is essentially controlled by environmental temperature and that photoperiod has no significant effect on given experimental conditions. A long term study, carried out during two consecutive years leads to similar results (Vivien-Roels et al. 1984).

Also in amphibians, breeding activity is strongly influenced by environmental temperature sometimes associated with photoperiod (Lofts, 1974, 1975). For example in the green frog, Räna esculenta it has been reported that temperature is the primary proximate factor in the regulation of quiescence, whereas stimulation and maintainance of active spermatogenesis requires both high temperature and "normal" photoperiod (Rastogi and Iela, 1976).

In fishes, a recent review of Crim (1982) reports that both light and temperature variations cue seasonal gonad development in the teleosts, the relative importance of these two factors differing among the various fish species. In the european minnow, Phoxinus phoxinus, for example, to attain full maturity for both sexes requires a combination of long days and high temperatures; without this combination maturation of the eggs in the ovary is arrested and spermatogenesis in males is helded to the spermatocyte stage (Lofts, 1975).

Some species such as the tench, Tinca tinca, or the cyprinid fish Migrogrex terrae-sanctae, respond primarly to changes in the temperature for regulating their reproductive cycles. On the other hand in some salmonids and gasterosteids, photoperiod seems to be the major environmental cue timing reproductive cycles (De Vlaming, 1972, Crim, 1982). But a same species frequently respond to both changes in photoperiod and tempera-

ture; in the trout for example, gonad maturating is stimulated by decreasing photoperiod although blood gonadotrophin levels are modified by temperature (Breton and Billard, 1977).

From this brief survey on the interactions of photoperiod and temperature in regulating gonadal cycles in non-mammalian vertebrates and more especially in poïkilotherms, it appears that these two factors are strongly interdependent and act concurrently, at least in species living in high or mid latitudes.

Animals needs thus to differenciate more than one environmental information. Since the pineal gland is now well-known to be involved in mediating the effects of photoperiodic information on seasonal reproduction (ref. in Reiter 1981b, Goldman, 1983, Vivien-Roels and Pévet, 1983), this raises the question if the pineal is able to integrate more than one information and how it integrates these different informations.

THE PINEAL GLAND AND THE INTEGRATION OF PHOTOPERIOD AND ENVIRONMENTAL TEMPERATURE IN NON-MAMMALIAN VERTEBRATES.

To date, two groups of compounds have been identified and proposed to be responsible of the endocrine capabilities of the pineal gland of vertebrates : indoleamines, especially 5-methoxyindoles, and peptides which are not yet fully identified.

Indoleamines have received more attention, since, at least the synthesis of serotonin and by consequence of serotonin derivatives, such as melatonin, is controlled by light (ref.in Wurtman et al.1968,Reiter,1981a) . On the other hand it is now well-known that pineal indoles fluctuate diurnally and that their rhythmicity is clearly linked to the recurring cycle of light and darkness(Wurtman et al.1968).In all species studied to date, pineal serotonin concentrations are high during day-time, while pineal melatonin concentrations strikingly show a peak in the dark phase. Nevertheless in some species the amplitude of the day-night rhythm of serotonin as well as melatonin may vary with seasons (ref. in Vivien-Roels and Pévet, 1983). Especially in species living in temperate regions, where pho-

toperiod and external temperature largely fluctuates throughout the year, temporal relationships have been reported between the seasonal variations in pineal or circulating indole concentrations and the annual variations in daylength or environmental temperature. These results have suggested that the pineal gland of vertebrates could integrate variations in these two environmental factors "via indole derivatives" (ref. in Vivien-Roels, 1983, Vivien-Roels and Pévet, 1983). Moreover there are now some preliminary results concerning the effects of these two factors on the day-night or seasonal rhythms in pineal indole derivatives.

Photoperiod

The possible influence of photoperiodic changes in the day-night or seasonal production of indoles derivatives has only been investigated in a few species to date.

In birds, Cockrem and Follett (1983) recently reported that photoperiod influences the duration and the amplitude of the night peak of melatonin in the pineal of the quail, Coturnix coturnix japonica. Using two light-dark (LD) cycles, of respectively LD : 8-16 and LD : 16-8, they reported that the duration of elevated melatonin levels depends on the duration of the night, but the amplitude is lower under LD : 8-16 than under LD 16-8. Similar results had already been reported for N-Acetyltransferase activity (NAT) in the chicken (Binkley et al., 1977) and in the djungarian hamster, Phodopus sungorus (Hoffmann, 1981); maximal values being higher during long photoperiod (LD : 16-8), but duration of elevated NAT activity being distinctly longer in short days (LD, 12-12, LD : 8-16). Also Florant and Tamarkin (1984) just report that in the marmot, Marmota flavinensis, the mean duration of elevated night melatonin concentrations was significantly longer in short photoperiods (LD : 4-20, LD : 8-16) than in long photoperiods (LD : 16-8).

Photoperiods shorter (8L-8D) or longer (18L-18D) than 24 hours have also been shown to entrain the day-night rhythm of NAT activity (Doï et al., 1983).

In poïkilotherms data are very scarce to date. Menaker

and Wisner have recently demonstrated that "in vitro" the diurnal pineal melatonin rhythm may be entrained by a light-dark cycle in a lizard, Anolis carolinensis. (Menaker and Wisner 1983)

In the tortoise, Testudo hermanni, preliminary results have shown that experimental changes in photoperiod in winter (January-February) or in summer (June-July) did not significantly affect the day-night rhythm of melatonin concentrations in the pineal or in the plasma (Vivien-Roels and Arendt, 1981, 1983). More recently, in a long term study, male tortoises have been maintained during two different years under a constant long photoperiod (LD : 16-8) and normally changing temperature. Pineal melatonin concentrations, evaluated by radioimmunoassay (RIA) every 6 weeks or two months (Vivien-Roels et al. 1984) during day- and night-time, did not significantly differ from those of control animals maintained under natural conditions of photoperiod and temperature (Table 1). The night rise of melatonin was suppressed in both groups from November until March. Nervetheless in December 1983 and February 1984, in both controls and experimental group pineal melatonin concentrations were high during day-time. Higher melatonin concentrations during day-time were already observed previously in the plasma of tortoises killed in December 1977 (Vivien-Roels and Pévet, 1983). Although these results are difficult to interpreté at the moment, whithout further investigations, they suggest a number of hypotheses. First of all one cannot exclude the possibility of melatonin synthesis during day-time in some species or in some environmental conditions; may be also that animals, which are hibernating and burying during winter, do not detect the photoperiodic conditions and are "free-runing", and pineal melatonin rhythm may persist with a period slightly different from 24 hrs; but one has also to consider the possibility of a cross-reaction with other indole derivatives synthetized during day-time.

What appears nevertheless clearly from these results is that photoperiod has no significant effect on the amplitude of the day-night production of pineal melatonin in the tortoise. A possible effect of photoperiod on the duration of the night peak of melatonin has now to be tested in this species.

1981-1982

	Time of autopsy	July	September	October	December	February	march	may	june
Controls	12 h					20∓4	46∓10	16∓2	
	24 h					41∓13	23∓10	50∓17	
LD:16-8	12 h					30∓14	13∓3	28∓8	
	24 h					14∓2	23∓10	90∓21	
T°:25-30°C	12 h					13∓2	221∓3	12∓1	
	24 h					110∓40	657∓200	601∓64	

1983 - 1984

Controls	12 h	26∓5	100∓21	71∓29	268∓38	157∓62	41∓7	110∓24	68∓14
	24 h	295∓101	97∓24	124∓20	43∓5	65∓6	303∓162	284∓39	298∓88
LD:16-8	12 h	51∓10	82∓6	55∓15	278∓65	381∓100	23∓5	42∓27	
	24 h	494∓125	127∓36	258∓91	50∓9	250∓152	92∓29	301∓47	
T°:25-30°C	12 h	33∓7	142∓37	23∓3	110∓31	138∓95	38∓4	75∓15	134∓59
	24 h	440∓103	385∓72	475∓178	362∓58	570∓140	405∓163	731∓235	399∓158

Table 1 : Day and night pineal melatonin concentrations (pg/mg gland), in animals maintained during the whole year, under natural conditions of photoperiod and temperature (controls) under constant long photoperiod (16L-8D), or under constant high temperature (25-30°C). (Data obtained in collaboration with Drs. J.Arendt and M. Fèvre-Montange).

Environmental temperature

Data on the effects of environmental temperature on indole metabolism in non-mammalian vertebrates are very scarce and limited to reptiles to date.

In a lizard, Sceloporus occidentalis, Quay et al. (1971) have reported that pineal Hydroxy indole-O-methyltransferase (HIOMT) activity was lower in animals collected during cold days (12.5-18°C) than during warm days (20-25°C). Also in the green sea-turtle Chelonia mydas and in the loggerhead turtle, Caretta caretta, Owens and Gern (1981) recently reported that serum melatonin concentrations were higher in animals kept at 31°C than in those kept at 23°C. In the tortoise, Testudo hermanni, environmental temperature appears to be an important factor regulating the amplitude of the day-night production of pineal melatonin. An increase in the environmental temperature in winter induces an important and significant increase in the night production of pineal melatonin, while a decrease of the environmental temperature in summer abolishes the night peak of pineal as well as circulating melatonin (Vivien-Roels and Arendt 1981, 1983). In a long term study, male tortoises have been maintained under a constant high temperature (25-30°C) and normal changing photoperiod during two different years (Table 1). In such animals, a night peak in pineal melatonin remains present all along the year, while in controls the night peak of melatonin is abolished from November until March. Such animals also remain sexually active throughout the year, as evaluated by gonado-somatic index (GSI) and circulating testosterone concentrations (Vivien-Roels et al., 1984). These results clearly show that temperature is an important factor in regulating the day-night production of melatonin in the pineal gland of the tortoise.

The importance of environmental temperature on the amplitude of the night rise of pineal melatonin has been recently confirmed in a lizard, Anolis carolinensis by Menaker and Wisner (1983). These authors, using a superfusion technique, have reported a clear tendency for the amplitude of the day-night rhythm of melatonin production to be greater at 27-37°C than at 22°C.

In other vertebrates, temperature has been reported to affect HIOMT or NAT activity in mammals (Nir et al., 1975, 1978, Ulrich et al., 1973). Recently, Vanecek et al. (1984) have demonstrated a clear effect of environmental temperature on the amplitude of the day-night rhythm in pineal melatonin concentrations in the golden hamster, Mesocricetus auratus. These authors reported a marked daily rhythm in pineal melatonin content in warm-adapted hamsters (25°C) while in cold-adapted hamsters, hibernating for at least 2 days no rhythm in pineal melatonin could be observed. These results corroborate our previous observations in the tortoise (Vivien-Roels et al., 1979) that during winter and hibernation, the day-night rhythm in pineal melatonin was abolished.

When overlooking the literature, it appears that, depending on the species, photoperiod as well as environmental temperature, both intervene in the regulation of the day-night or seasonal variations in pineal indole metabolism. All the data available to date, concerning the effects of photoperiod and temperature suggest that the pineal gland is able to integrate these two environmental factors "via indole derivatives", but they also open several questions.

Are the different indole derivatives implicated in the integration of a given stimulus, as already suggested a few years ago by Klein and Weller (1972). Studying the effects of light-temperature regimes on pineal serotonin, HIOMT and NAT content, these authors concluded that there could be "a dual mechanism controlling pineal function in the suckling rat", NAT being sensitive to temperature and serotonin to environmental illumination. Also our results concerning the effects of photoperiod and temperature on serotonin and melatonin rhythms in the tortoise are in agreement with such an hypothesis. But on the other hand, data obtained in some reptiles, birds and also in mammals suggest that photoperiod could regulate the duration of the night rise in pineal melatonin while temperature could regulate the amplitude of the day-night rhythm. The different results obtained in the different species also open

the question if the relative importance of the different indole derivatives in mediating the effect of a given environmental factor may vary from class of vertebrate to another.

To date, it is not possible to answer these different questions but it is now well-known that several hydroxyindoles can be converted to methoxyindoles by the enzyme HIOMT. In a recent review, Balemans (1981), reported that HIOMT appears to be the synthetizing enzyme for a least six 5-methoxyindoles (MI) : 5-methoxytryptophane (MW), 5-methoxytryptamine (MT), melatonin (aMT), 5-methoxytryptophol (ML), 5-methoxyindole acetic acid (MA) and O-Acetyl 5-methoxytryptophol. All of these compounds are potentially pineal active compounds. During the last few years, more attention has been paid to the day-night and seasonal variations of the different 5-methoxyindoles synthetized by the pineal gland of vertebrates and their possible role in regulating seasonal reproduction (ref. in Pévet, 1983).

DIFFERENT 5-METHOXYINDOLES SYNTHETIZED IN THE PINEAL GLAND IN NON-MAMMALIAN VERTEBRATES : DAY-NIGHT AND SEASONAL VARIATIONS.

Day night and seasonal rhythms in the synthesis of the different 5-methoxyindoles are now available in a few species of non-mammalian vertebrates.

In birds, recent data (Grady et al., 1984), using high-performance liquid chromatography with electrochemical detection, report day-night differences of different hydroxy- and methoxyindoles in the pigeon, Columba livia, killed in mid-june. Melatonin and N-acetyl serotonin were reported to be 3-fold increased during night, while serotonin, 5-hydroxyindole acetic acid and MA were decreased at night and ML did not show day-night differences. Previous results of Balemans et al. (1981) in the parakeet, Melopsittacus undulatus, had reported that the HIOMT activity responsible for the synthesis of aMT/ML shows a maximal activity during the dark phase in April as well as in November, while MW and MT synthesis were high during day-time, but with lower synthesis in november than in april for MT. More recently, Collin et al. (1981) in the same species reported that in autumn, among the MI, aMT/ML synthesis was the most important.

In reptiles, the day-night synthesis of 5-methoxyindoles has been evaluated in the pineal of the tortoise, Testudo hermanni Gmelin, using the technique of Balemans et al. (1978). Animals, maintained in a natural environment, were killed every 4 hours during 24 hours, at different times of the seasonal reproductive cycle (July, October, January and April). When comparing the relative importance of the different 5-methoxyindoles (MI) synthetized by the pineal (fig. 1) it appears that ML is, by far, the most abundantly MI synthetized in this species. Such results need however to be confirmed by other techniques.

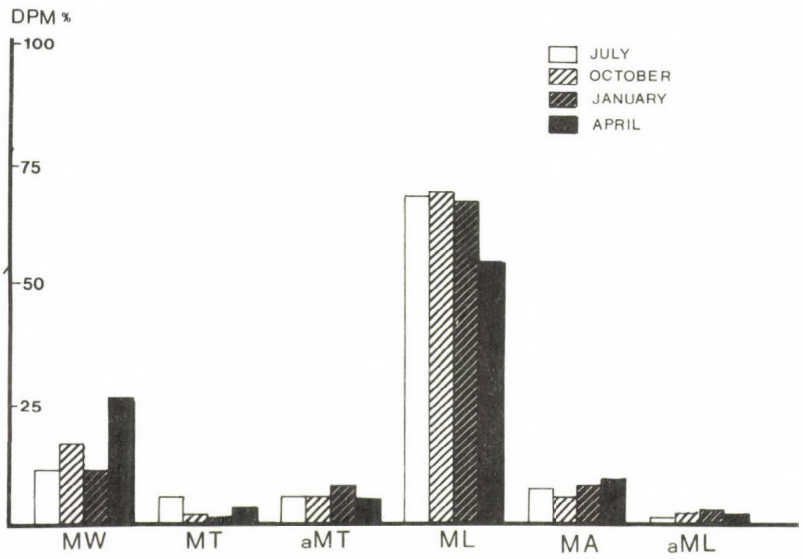

Fig. 1 : Percentage of the different 5-methoxyindoles synthetized in the pineal gland of the tortoise, Testudo hermanni, over a period of 24 hours, at different times of the annual reproductive cycle (data of Vivien-Roels and Balemans).

In cyclostomes, day-night variations in the synthesis of MI have been reported in the lamprey, Lampetra planeri, killed in October (Meiniel et al., 1983). In this species MT appears to be quantitatively the most important MI (60 % of the different MI), aMT representing only 2.8 %. Day-night variations of synthesis are only reported for MT and aML, aML being synthetized during day-time and MT at the beginning of the dark period.

When comparing the different results obtained in the different species and in different seasons, it appears that the relative importance of the different MI synthetized by the pineal, as well as the different diurnal rhythms vary considerably from one species to another, suggesting that not only melatonin, but also other MI may be of physiological importance.

In the species where it has been investigated, seasonal variations in the day-night synthesis of MI are present, showing temporal relationships with the annual changes in daylength and environmental temperature as well as with the seasonal reproductive cycle, suggesting that not only melatonin, but also other MI could be implicated in mediating the effects of environmental factors on seasonal reproduction. Experimental investigations on the effects of different MI on seasonal gonadal activity are now needed to test such a hypothesis (see : Pévet 1983).

To date, data concerning the influence of indole derivatives in mediating the effects of environmental factors on seasonal reproduction in non-mammalian vertebrates are essentially limited to melatonin.

MELATONIN AND SEASONAL REPRODUCTION IN NON-MAMMALIAN VERTEBRATES.

There are now several data in the literature, reporting that the pineal gland of non-mammalian vertebrates is involved in long term adaptation of seasonal reproduction and that melatonin may be implicated in this phenomenon (ref. in Ralph, 1978, De Vlaming and Olcese, 1981, Vivien-Roels and Pévet, 1983).

In fishes, the effects of melatonin injections on seasonal gonadal activity have been studied in various species and have been recently reviewed by De Vlaming and Olcese (1981). On the basis of the fact that in many fishes, the effect of melatonin appears to be photoperiod-dependent, and that at a given photoperiod the effects on gonads may vary with the time of the year, these authors suggested that in fishes "melatonin mediates the gonadal regressive effect of declining daylength during late summer, the inhibitory effects of short daylengths during fall and winter, and/or retards rapid gonadal growth in daylength is increasing". In the three-spined stickelback, Gasterosteus aculeatus, Borg and Ekström (1981), investigated the effects of melatonin injections on male and female sexual maturation during different seasons; high doses of melatonin excerted antigonadal effects in males and females in winter, at high temperature, while a slight progonadal effect was reported in females with low melatonin doses at the end of the breeding season.

In amphibians most studies concerning the effects of melatonin injections did not specify photoperiodic or temperature conditions, time of the year, or injection time (ref. in De Vlaming and Olcese, 1981). Recently Delgado et al. (1983) reported that melatonin injected at the begining of the dark phase induced a significant regression of the gonads in female frogs, Rana ridibunda, housed in long photoperiod (18L-6D) and high temperature in November. Similar results had been previously obtained in the frog, Hyla cinearea, showing inhibiting effects of melatonin injections in winter, in long photoperiod (16L-8D) and high temperature.

In reptiles, most results have been obtained by Haldar-Misra and Thapliyal (1981a,b) in the lizard, Calotes versicolor. In this species melatonin injections in late afternoon inhibit the gonadal activity during the progressive, reproductive and early regression phases. Similar results had been previously reported in another lizard, Callisaurus draconoides (Packard and Packard, 1977). Also Underwood (1981), in Anolis carolinensis, demonstrated a seasonally dependent effect of melatonin injections on the photoperiodic gonadal response in this species. Preliminary results obtained in the tortoise, Testudo hermanni, maintained in a natural environment show that subcutaneously melatonin "silastic" capsules implanted in July, during the period of sexual activity, induced after six weeks a decrease of both the gonado-somatic index and circulating testosterone concentrations (Vivien-Roels, unpublished data).

Data concerning effects of melatonin on seasonal reproduction in birds are relatively scarce. A recent review of Ralph (1981) concerning the role of pineal gland in avian reproduction showed that results were very inconsistent; but most data concern chickens, quails or ducks which are highly domesticated species. Nevertheless in a series of experiments in white leghorn chickens, Balemans et al. (1977a, b) it has been reported that melatonin is generally inhibitory for sexual development, while ML was reported to be stimulatory to sexual development in juvenile chickens but inhibitory in maturing or adult chickens.

As in mammals (ref. in Reiter 1981b, Hoffmann 1981), in non-mammalian vertebrates, melatonin effects appear to be dependent on the species, the season and the environmental conditions to which animals are exposed. In some species, as recently suggested by Ralph (1983) "the modulation of the daily pattern of melatonin secretion across the season by photoperiods of progressively changing durations may be used by many vertebrates to cue annual events". In other species, such as the tortoise for example, melatonin could be implicated in the mediation of temperature effects on seasonal reproduction.

CONCLUSION AND OUTLOOK

From the different data obtained in various species, it appears clearly that, as in mammals, in non-mammalian vertebrates, the pineal gland is involved in the integration of environmental factors such as photoperiod and environmental temperature, and that various indole derivatives may be implicated in such phenomenons.

Nevertheless, as often the case, when overviewing the literature, all these results raise many questions.

It has been recently suggested by Pévet and Haldar-Misra (1982), Pévet(1983), that "all methoxyindoles" -and probably also hydroxyindoles-" could be implicated in a system enabling the pineal and some other structures of the brain, to perceive, to differenciate and to integrate environmental factors". (see also Pévet this volume).

If the different indole derivatives are implicated in the integration of a given factor or not has now to be answered. To date, it is well-known that melatonin, which has been the most studied compound, is deeply implicated in conveying photoperiodic information, but in some species, especially poïkilotherms, melatonin also is able to integrate variations in environmental temperature. Also recent results of Semm et al.(1982) reported that in the rat, the activity of NAT and correspondingly of the melatonin content of the pineal gland during night were strongly depressed following the exposure to an altered magnetic field.

On the other hand, melatonin is known to be also present in the retina of various vertebrates (Bubenik et al. 1974, Vivien-Roels et al. 1981) and has recently been demonstrated in invertebrate's eyes (Vivien-Roels et al. 1984).
This raises the question of the physiological significance of melatonin. As suggested by Gern and Karn (1983) "melatonin synthesis appears as a common attribute of ciliate photoreceptor cells and may be a fairly consistent phylogenetic feature..The nocturnal pulse of melatonin would be ideal for daily or annual timing functions" such as seasonal reproduction for example. In such a concept, the duration of the night rise in pineal

melatonin could be controlled by photoperiod, while its amplitude could be controlled by other factors such temperature, magnetic field, rainfall...Comparative investigations are now needed to test such an hypothesis.

In addition to melatonin, the physiological importance of the other indole derivatives, such as serotonin, MT or ML has also to be investigated in the near future. The recent development of new techniques such as HPLC with electrochemical detection, or GCMS will be helpfull in studying these compounds.

To date, many questions remain still open, but it appears nevertheless that the pineal gland of vertebrates is primary concerned with the synchronization of central functions, such as seasonal reproduction for example, with changes in environmental conditions. Comparative field studies in animals living in different ecological conditions and laboratory studies in animals maintained in experimental conditions of environmental factors are now needed to clarify the mechanisms by which the pineal gland and the indole derivatives integrate and mediate the effect of environmental factors.

REFERENCES

BAKER J.R., 1938. The evolution of breeding seasons. In : G.R. de Beer (ed.) Evolution, Essays on aspects of Evolutionnary biology. Oxford University Press, 161-177.

BALEMANS M.G.M., 1981. Indole metabolism in the pineal gland. The harderian gland and the retina of mammals. In " A. Oksche and P. Pévet (eds.) The Pineal Organ : Photobiology-Biochronometry-Endocrinology. Developments in Endocrinology 14, 261-279.

BALEMANS M.G.M., VEERDONK M.G.M. and KAMER v.d. J.C., 1977a. The influence of 5-methoxytryptophol, a pineal compound, oncomb growth, ovarian weight, follicular growth and egg production of juvenile, maturing and adult white leghorn (Gallus domesticus L.) J.Neural Transm. 41(1), 37-46.

BALEMANS M.G.M., VEERDONK v;d; F.G.C. and KAMER v.d. J.C, 1977b. Effect of injecting 5-methoxyindoles, pineal compounds, on testicular weight of white leghorn cokerels (Gallus domesticus L.) J. Neural Transm. 41(1)47-57.

BALEMANS M.G.M., BARY F.A.M., LEGERSTEE W.C. and VANBENTHEM J., 1978. "Estimation of methylating capacity in pineal gland of Rat with special reference to methylation of N-Acetyl-serotonin and 5-methoxytryptophol separately". Experientia, 34, 1434-1435.

BALEMANS M.G.M., COLLIN J.P., LEGERSTEE W.C. and JUILLARD M.T., 1981. Preliminary investigations on the circadian rhythmicity of methylation of 5-hydroxyindoles in the pineal gland of the parakeet . Biol. Cell. 42, 167.

BINKLEY S., STEPHENS J.L., RIEBMAN J.B. and REILLY K.B., 1979. "Regulation of pineal rhythms in chicken : Photoperiod and dark-time sensitivity".Gen.Comp.Endocrinol. 32(4),411-416.

BORG B. and EKSTRÖM P., 1981. Gonadal effects of melatonin in the three spined stickle-back, Gasterosteus aculeatus L. during different seasons and photoperiods. Reproduction nutrition development, 21,6A,919-928.

BRETON B. and BILLARD R., 1977. Effects of photoperiod and temperature on plasma gonadotrophins and spermatogenesis in the rainbow trout, Salmo gairdnerii Richardson. Ann. Biol. Anim. Bioch. Biophys. 17, 331-340.

BUBENIK G.A., BROWN G.M., UHLIR I. and GROTA L.J., 1974. "Immunohistological localization of N-acetylindolealkylamines in pineal gland, retina and cerebellum". Brain research, 81(2) 233-242.

CALLARD I.P. and HO S.M., 1980, Seasonal reproductive cycle in reptiles. In : Reiter R.J. and Follett B.K. (eds.) Seasonal Reproduction in highter vertebrates. Progress in reproductive Biol., 5P, 5-38.

COCKREM J.F. and FOLLETT B.K., 1983. "Rhythms of melatonin in the quail pineal gland in vivo and in vitro". Abstract XIIth Conference of European comparative endocrinologists. Sheffield 31 july-5 august 1983. p178.

COLLIN J.P., BALEMANS M., JUILLARD M.T., LEGERSTEE W.C., VANBEBTHEM J. and VOISIN P., 1982. Indole metabolism at the cellular level : an in vivo combined radiobiochemical and high resolution autoradiographic study in the avian pineal with special reference to melatonin/5-methoxytryptophol. Biology of the Cell, 44(1), 25-34.

CRIM L.W., 1982. Environmental modulation of annual and daily rhythms associated with reproduction in teleost fishes. Canad. J. Fish aquat. Sci. 39, 17-21.

DELGADO M.J., GUTIERREZ P. and ALONSO-BEDATE M., 1983. Effects of daily melatonin injections on the photoperiodic gonadal response of the female frog Rana ridibunda. Comp. Biochem. Physiol. 76(2), 389-392.

DOI O., KOYAMA E., NAKAMURA T. and TANABE Y., 1983. Photoperiodic regulation of serotonin N-acetyltransferase activity in the pineal gland of the chicken. Comp. Biochem. Physiol. 74(2), 195.

FLORANT G.L. and TAMARKIN L., 1984. Plasma melatonin rhythms in eutheric marmots, Marmota flaviventris. Biol. Reprod. 30, 332-337.

FOLLETT B.K. and FOLLETT D.E. (Eds.), 1981. Biological clocks in seasonal reproductive cycles. Bristol Scientechnica, 1-292.

GERN W.A. and KARN C.M., 1983. Evaluation of melatonin's function and effects. In R.J.Reiter (ed.) Pineal research review, Alan Liss Inc. New-York, 1, 49-80.

GOLDMAN B.D., 1983. The physiology of melatonin in mammals. In R.J. Reiter (ed.) Pineal research Review. Alan R. Liss, Inc., New-York, 1, 145-182.

GRADY R.K., CALIGURI A. and MEFFORD I.N., 1984. Day-night differences in pineal indoles in the adult pigeon (Columba livia). Comp. Biochem. Physiol. 78C,141-144.

HALDAR-MISRA C. and THAPLIYAL J.P., 1981a. Responses of Reptilian Gonad to Melatonin. Neuroendocrinology 33(6),328-332.

HALDAR-MISRA C. and THAPLIYAL J.P., 1981b. Effect of melatonin on the testes and the renal sex segment in the garden lizard, Calotes versicolor. Canad. J. Zool.,59(1), 70.

HOFFMANN K., 1981. Photoperiodic function of the mammalian pineal organ. In A.Oksche and P.Pévet (eds.) The Pineal Organ, Photobiology, Biochronometry, Endocrinology. Elsevier North-Holland Biomedical Press. Developments Endocrinol. 14, 123-138.

KAPPERS J.A. and PEVET P. (eds.), 1979. The pineal gland of Vertebrates including man. Progr. Brain Res. 52. Amsterdam, Elsevier/North Holland Biomedical Press.

KLEIN D.C. and WELLER J.L., 1970. Indole metabolism in the pineal gland : a circadian rhythm in N-acetyltransferase. Science, 169, 1093-1095.

KUCHLING G., 1982. Effect of temperature and photoperiod on spermatogenesis in the tortoise Testudo hermanni hermanni Amphibia Reptila 2, 329-341.

LICHT P., 1972. Environmental physiology of reptilian breeding cycles : role of the temperature. Gen. Comp. Endocrinol. suppl.3,477-488.

LOFTS B., (Ed.), 1974. Physiology of the amphibian, Vol. II Reproduction. Academic Press.

LOFTS B., 1975. Animal Photoperiodism. The Institute of Biology's studies in E. Arnold (ed.) Biology n°25, 1-64.

MEINIEL A., VANBENTHEM J., MEINIEL R., PEVET P. and BALEMANS M.G.M., 1983. Day and night rhythmicity in the capacity of the pineal complex of the ammocete of Lampetra planeri (Petromyzntids) to synthetize different 5-methoxyindoles. Neuroendocrinol. letters, 5, 415.

MENAKER M. and WISNER S., 1983. Temperature compensated circadian clock in the pineal of Anolis. Neurobiology, 80, 6119-6121.

NIR I., HIRSCHMANN N. and SULMAN F.G., 1975. The effects of heat on rat pineal hydroxyindole-O-methyltransferase activity. Experientia, 31(7), 867-868.

NIR I. and HIRSCHMANN N., 1978. Pineal N-acetyltransferase depression in rats exposed to heat. Experientia 34,1645-46.

OKSCHE A. and PEVET P. (eds.), 1981. The Pineal Organ : Photobiology-Biochronometry-Endocrinology. Dev. Endocrinol.14, Amsterdam.Elsevier/North-Holland. Biomed. Press.

OWENS D.W. and GERN W.A., 1981. The pineal gland and melatonin in sea-turtles. Abstract Ninth International symposium of comparative endocrinology. Hong-Kong 7-11 dec.1981.,232.

PACKARD M.J. and PACKARD G.C., 1977. Antigonadotrophic effect of melatonin in male lizards (Callisaurus draconoides) Experientia, 33, 1665-1566.

PERRY J.S. and ROWLANDS I.W. (Eds.). The environment and reproduction in mammals and birds". Blackwell Scientific publications, Oxford, London, Melbourne. J. Reproduction and fertility, suppl.19, 1-613.

PEVET P., 1979. Secretory processes in the mammalian pinealocyte under natural and experimental conditions. In J.A. Kappers and P. Pévet (eds.) The Pineal gland of Vertebrates including man. Elsevier/North-Holland Biomed. Press, Progr. Brain Res., 52, 149-194.

PEVET P., 1983. Is 5-methoxytryptamine a pineal hormone ? Psychoneuroendocrinology, 8, 61-74.

PEVET P. and HALDAR-MISRA C., 1982. Effect of 5-methoxytryptamine on testicular atrophy induced by experimental or natural short photoperiod in the golden hamster (Mesocricetus auratus). J. Neural Transm. 55, 69-84.

QUAY W.B., 1965. Indole derivatives of pineal and related neural and retinal tissues. Pharmacol.rev. USA, 17(3) 321-345.

QUAY W.B., STERBINS R.C., KELLY T.D. and COHEN N.W., 1971. Effect of environmental and physiological factors on pineal acetylserotonin methyltransferase activity in the lizard, Sceloporus occidentalis. Physiol.Zool.,44,241-248.

RALPH C.L., 1975. The pineal gland and geographical distribution of Animals. Int. J. Biometeorol. 19(4), 289-303.

RALPH C.L., 1978. Pineal control of Reproduction : non-mammalian Vertebrates. In R.J.Reiter (ed.) The pineal and reproduction. Progr. Reprod. Biol., 4, 30-50.

RALPH C.L., 1983. Evolution of pineal control of endocrine function in lower vertebrates. American Zoologist 22, (3), 597-606.

RASTOGI R.K., IELA W., SAXENA P.K. and CHIEFFI G., 1976. The control of spermatogenesis in the Frog, Rana esculenta. J. Exp. Zool., 196 (2), 151.

REITER R.J. (ed.), 1978. Interaction of photoperiod, pineal and seasonal reproduction as exemplified by findingd in the Hamster. In The pineal and reproduction. Progr. Reprod. Biol., 4, 169-190.

REITER R.J.(ed.), 1981a. The pineal gland. vol.I. Anatomy and biochemistry. CRC Press. Boca Raton, Florida

REITER R.J.(ed.), 1981b. The pineal gland. vol.II. Reproductive effects. CRC Press. Boca Raton, Florida.

ROWAN W., 1925. Relation of light to bird migration and developmental changes. Nature (Lond.), 115, 494-495.

SEMM P., SCHNEIDER T., VOLLRATH L. and WILTSCHKO W., 1982. Magnetic sensitive pineal cells in Pigeons. In Papi and Walraff (eds.) Avian Navigation, Springer Verlag, Berlin, 329-337.

UNDERWOOD H., 1981. Effect of pinealectomy and melatonin on the photoperiodic gonadal response of the male lizard Anolis carolinensis. J.Exp.Zool. 217(3), 417-422.

UNDERWOOD H. and HALL D., 1982. Photoperiodic control of Reproduction in the male lizard, Anolis carolinensis. J. Comp. Physiol., 4(A), 485-492.

ULRICH R., YUWILLER A., WETTERBERG L. and KLEIN D., 1973. Effects of light and temperature on the pineal gland in suckling rats. Neuroendocrinology 13(4-5),255-263.

VANECEK J., JANSKY L., ILLNEROVA H. and HOFFMANN K., 1984. Pineal melatonin in hibernating and aroused golden hamsters (Mesocricetus auratus), Comp. Biochem. Physiol. 77(A), 759-762.

VIVIEN-ROELS B., 1981. Pineal control of reproduction in non-mammalian Vertebrates. In A.Oksche and P.Pévet (eds.) The pineal organ : photobiology-biochronometry-endocrinology.Developm. Endocrinol. 14, 315-334.

VIVIEN-ROELS B., 1983. The pineal gland and the integration of environmental information:possible role of hydroxy and methoxyindoles. Molecular Physiology, 4(5-6), 331.

VIVIEN-ROELS B. and ARENDT J., 1981. Relative role of environmental factors photoperiod and temperature in the control of serotonin and melatonin circadian variations in the pineal organ and plasma of the tortoise Testudo hermanni Gmelin. In N.Birau and W.Schloot (eds.) Adv. in Biosciences 29, 401-406.

VIVIEN-ROELS B. and ARENDT J., 1983, How does the indoleamines production of the pineal gland respond to variations of the environment in a non-mammalian vertebrate Testudo hermanni Gmelin. Psychoneuroendocrinol.8(3),327-332.

VIVIEN-ROELS B. and PEVET P., 1983. The pineal gland and the synchronization of reproductive cycles with variations of the environmental climatic conditions with special references to temperature. In R.J.Reiter (ed.) Pineal Research Reviews, Alan R.Liss,Inc. New-York,1,91-144.

VIVIEN-ROELS B., ARENDT J. and BRADTKE J., 1979. Circadian and circannual fluctuations of pineal indoleamines (serotonin and melatonin) in Testudo hermanni Gmelin (Reptilia Chelonia) I. under natural conditions of photoperiod and temperature. Gen. comp. endocrinol., 37, 197-210.

VIVIEN-ROELS B., PEVET P., DUBOIS M.P., ARENDT J. and BROWN G.M. 1981. Immunohistochemical evidence for the presence of melatonin in the pineal gland, the retina and the harderian gland. Cell. Tissue Res. 217, 105-115.

VIVIEN-ROELS B., FEVRE-MONTANGE M. and ARENDT J., 1984. Long term effect on environmental factors on pineal and gonadal function in the male tortoise Testudo hermanni Gmelin Abstract, Satellite symposium "Pineal 84" 7th Internat. Congress of Endocrinology, Digby,Nova Scotia,Canada July 8-10 1984.

VIVIEN-ROELS B., PEVET P., BECK O. and FEVRE-MONTANGE F., 1984. Identification of melatonin in the compound eyes of an Insect, the locust (Locusta migratoria) by radioimmunoassay and gas chromatography -mass spectrometry. Neurosc. letters (in press).

VLAMING de V.L., 1975. Effects of photoperiod and temperature on gonadal activity in the Cyprinid teleost Notemigonus crysoleucas. Biol. Bull., 148, 402-415.

VLAMING de V.L., 1981. The pineal and reproduction in fish, amphibians and reptiles. In R.J.Reiter (ed.) The pineal gland vol.II reproductive effects. CRC Press. Boca Raton Florida, 1-30.

WELKER M.A., SEMM P. and VOLLRATH L. (1982) Effects of an artificial magnetic field on the secretory activity of the rat pineal gland. Abstract 13a. Symposium German Endocrinol. Society. Salzburg, p.122.

WURTMAN R.J., AXELROD J. and KELLY D.E., 1968. The Pineal. Academic Press, New-York, London.

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

INTERACTION BETWEEN PHOTOPERIOD, PINEAL AND SEASONAL ADAPTION IN MAMMALS

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INTRODUCTION

In many mammals there is an annual cycle in reproduction and in other functions which is regulated by photoperiod, i.e. by the length of the daily light period and its seasonal changes (Hoffmann 1981a). It has been shown in several species that the pineal gland is involved in conveying the photoperiodic message onto the neuroendocrine axis. On the basis of the evidence available at the time of the last meeting of this society, the hypothesis was advanced that the temporal pattern of pineal melatonin synthesis and release is an integral part of this mechanism (Hoffmann, 1981b). In the meantime, more evidence has been published which strongly supports this hypothesis. Moreover, additional data have been provided which support the view that the pineal not only conveys inhibitory influences, but also participates in relaying stimulatory effects of photoperiod. In two mammalian species there is solid evidence that melatonin is involved in both the inhibitory as well as the stimulatory action of photoperiod. This evidence will be discussed. In addition, some information concerning the details of the photoperiodic action which have only recently been observed will also be presented. Since a substantial part of recent progress has been achieved in work with the Djungarian hamster (Phodopus sungorus), experiments using this species will mainly be presented, and will later be compared with results obtained in other photoperiodic mammals.

THE PHOTOPERIODIC SIGNAL

While there are many reports showing the effects of long and short photoperiods in different species of mammals, relatively little is known about what distinguishes these two external signals that have opposite effects. Only in two hamster species experiments investigating this question have been performed. In the golden hamster a marked 'critical' photoperiod of 12 1/2 h of light per day has been described, first by Gaston and Menaker (1967) and later, with more data, by Elliott (1974, 1976). Shorter photoperiods induced gonadal regression or maintained the regressed state, longer photoperiods caused rapid recrudescence

or maintained the active state of gonads. The critical photoperiod was independent of the previous light regime or of gonadal state.

In young Djungarian hamsters 35 days old, a similar critical photoperiod was found at 13 h of light per day (Hoffmann 1982a). In animals that had been raised in long photoperiods and which had large testes at this age, photoperiods of between 1 and 12 h of light per day induced gonadal regression, while photoperiods with 14 h of light or more further stimulated testicular growth (Fig. 1a). At 13 h of light, regression was observed in only half of the animals. In males that had lived from birth in short photoperiods and which, accordingly, had small and undeveloped testes at 35 days of age, 45 days of photoperiods between 1 and 12 h of light maintained the undeveloped state (Fig. 1b). Photoperiods between 14 and 16 h of light fully stimulated gonadal growth. At 13 h of light per day, there was only some stimulation. Thus, in general the picture resembled that found in animals coming from long photoperiods. However, there was one notable difference: Very long photoperiods, i.e. 20 h of light or constant light, were significantly less stimulatory than photoperiods with 14 to 16 h of light.

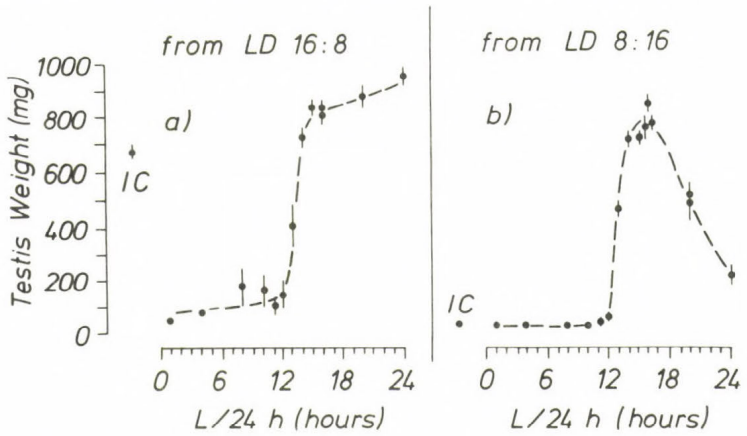

Fig. 1. Testis weight of young Phodopus that lived in long (a) or short (b) photoperiods for the first 35 days after birth, and were then maintained in the photoperiods indicated for another 45 days. Each point represents the mean ± SEM of 15 to 19 animals. IC = initial control at age 35. Note that the 'critical' photoperiod separating stimulatory from non-stimulatory photoperiods is the same for both pretreatments. From Hoffmann 1982a.

While these results in young Phodopus suggested that the critical photoperiod is not shifted by previous light conditions, in older animals a marked effect of the former schedule was found (Hoffmann 1984). In fully adult males that had been maintained from birth in 16 h of light per day, exposure to shorter photoperiods with 14 to 8 h of light caused gonadal regression (Fig. 2a). On the other hand, when animals of similar age were first

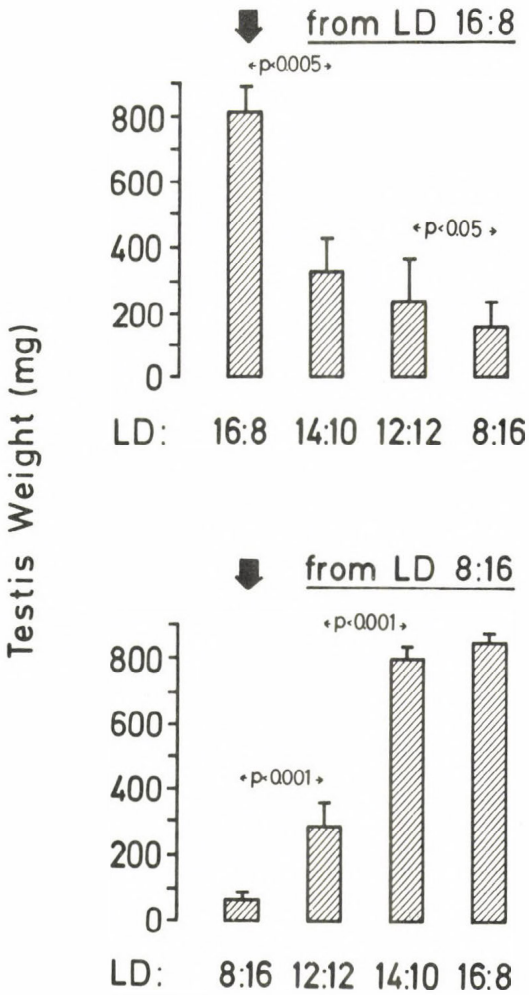

Fig. 2. Testis weight response to various photoperiods in fully mature Djungarian hamsters. Top: Testis weight after 77 days of exposure; hamsters had been maintained before in LD 16:8 for at least 6 months (from birth). Bottom: Testis weight after 52 days of exposure; animals had been kept in LD 8:16 for 12 weeks before and had regressed testes at the onset of treatment. Mean ± SEM of 10 animals/group. Black arrows indicate photoperiod at the start of the experiment. After Hoffmann 1984.

exposed to 8 h of light per day for 12 weeks, and then to photoperiods with between 12 and 16 h of light, these schedules induced recrudescence (Fig. 2b). These results show that in fully adult Djungarian hamsters the same photoperiod may be 'read' as long or short, depending on the previous light conditions. In

addition, they also show some influence ot the absolute length of the light-time. In animals coming from short photoperiods, 12 h of light stimulated significantly less than 14 or 16 h of light, i.e. the reaction was not all-or-none. Such results closely resemble those found in some bird species (Follet and Robinson 1980) and differ from those reported for the golden hamster.

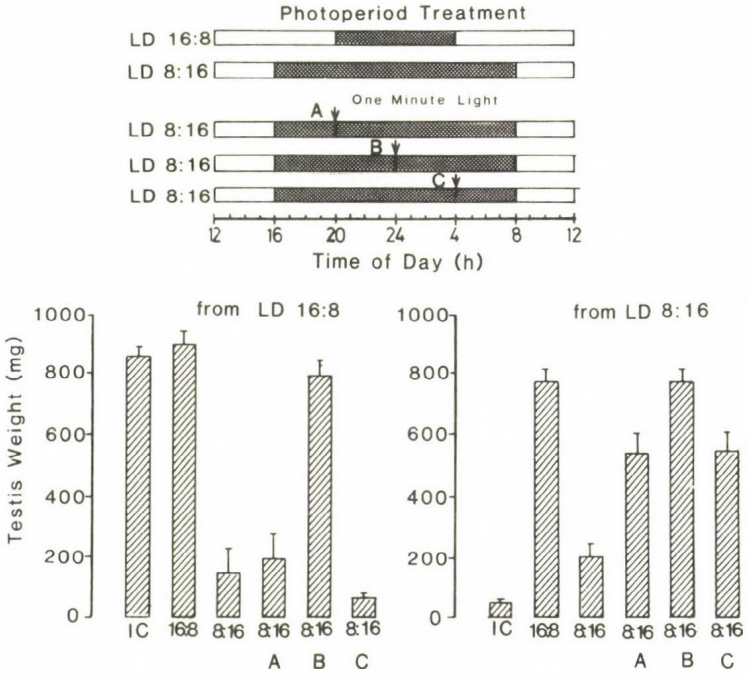

Fig. 3. Testis weight response to 'skeleton' photoperiods in fully mature Djungarian hamsters. Top panel: Diagram of the 5 photoperiodic schedules. One minute of light each night interrupted the dark phase in groups A, B and C at the times indicated by arrows. Left panel: Testis weight after 77 days of exposure in hamsters previously maintained in long days (LD 16:8). Right panel: Testis weight after 46 days of exposure in animals previously maintained in short natural days (LD 8:16). IC = initial controls before exposure. Means + SEM of 10 animals/group. Compare with Fig. 2. From Hoffmann 1982b.

The photoperiodic system in hamsters is based on a circadian mechanism of sensitivity to light (Elliot 1974, 1976). In order to be effective, photoperiods need not to consist of uninterrupted light periods. In the Djungarian hamster, interruptions of the dark phase (skeleton photoperiods) may fully mimic the effects of complete photoperiods (Hoffmann 1979, 1982b). An example is given in Fig. 3. It shows the effects of interruptions of the

dark phase by only 1 minute of light each night. The picture fully resembles the results illustrated in Fig. 2. If the long dark-time in short photoperiods is interrupted at midnight, leaving only 8 h of uninterrupted dark-time, the effect is the same as with long photoperiods (LD 16:8); it prevents regression or induces rapid recrudescence. If the dark interruption is located 4 h after light-off or 4 h before light-on, leaving 12 h of uninterrupted dark-time, testes of hamsters from long photoperiods regress, while in animal coming from short photoperiods there is recrudescence, though significantly less than when the dark period is interrupted at midnight. Pelage color and body weight show similar changes under these conditions (Hoffmann 1982b). In the golden hamster, similarly, effects of very brief interruptions of the dark-time have been reported (Ellis and Follet 1983, Earnest and Turek 1984). As little as 250 milliseconds of light showed some effect. Moreover, exposing the animals to brief light interruptions as infrequently as every 7 days had some effect, more frequent exposures were more potent.

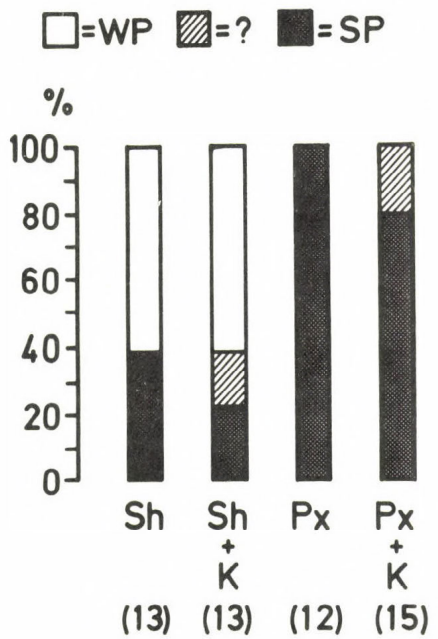

Fig. 4. Pelage color after 12 weeks in short photoperiods (LD 8:16). WP = winter pelage, ? = uncertain, SP = summer pelage. Sh = sham-pinealectomized, Px = pinealectomized, K = castrated. Number of animals in brackets. Sh versus Px differ significantly in both, gonad-intact and castrated hamsters, $p < 0.001$. Hoffmann, unpublished experiments.

PINEAL GLAND AND MELATONIN IN THE DJUNGARIAN HAMSTER

It has been shown in several experiments that in the Djungarian hamster, as in the golden hamster and in some other rodents, pinealectomy prevents the effects of exposure to short photoperiods (Hoffmann 1974, 1977, 1981b). This holds not only for the gonadal reaction but also for other functions that are influenced by photoperiod (Hoffmann 1977, 1978). Fig. 4 gives the results of a recent experiment on pelage color. Animals were

either pinealectomized or sham-operated before exposure to short photoperiods. Pinealectomy completely prevented the change into winter pelt, while most of the sham-operated hamsters changed color. This held for castrated as well as for gonad-intact animals, which shows that the change into winter pelage is not a secondary consequence of gonadal changes brought about by photoperiodic manipulation. Duncan and Goldman (1984) recently reported similar results.

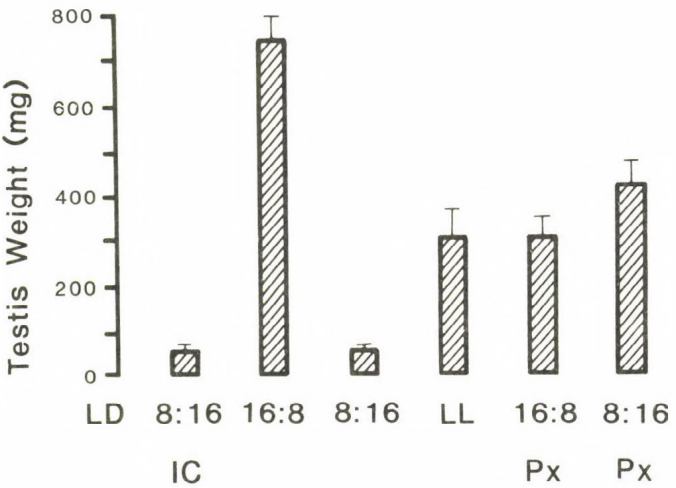

Fig. 5. Testis weight response of fully mature Djungarian hamsters after 47 days in the photoperiodic schedules indicated. Animals had lived in short photoperiods (LD 8:16) for 3 months before and, at the beginning of the experiment, had small and regressed testes as indicated by the initial controls (IC). Some of the hamsters were pinealectomized (Px) at this time. Note that in intact controls there is rapid recrudescence in LD 16:8, no recrudescence in LD 8:16 and moderate recrudescence in constant light (LL), while pinealectomized animals also show moderate recrudescence in both photoperiods. 10 to 12 animals per group. Values for intact hamsters in LL and pinealectomized hamsters in both photoperiods do not differ significantly, but each of these groups differs from intact animals in LD 16:8 ($p < 0.001$ in every case). Hoffmann, unpublished data.

In the Djungarian hamster there is also evidence that the pineal is involved, not only in conveying the effects of short photoperiods that induce gonadal regression as well as molt into winter pelage, but also in transducing the effects of long photoperiods which stimulate gonadal recrudescence and induce molt back into summer pelt (Hofmann and Küderling 1955, 1977, Brackmann and Hoffmann 1977, Hoffmann 1978). The results of a recent experiment showing the participation of the pineal in this process are presented in Fig. 5. Animals which had previously been maintained in short photoperiods and which, accordingly, had re-

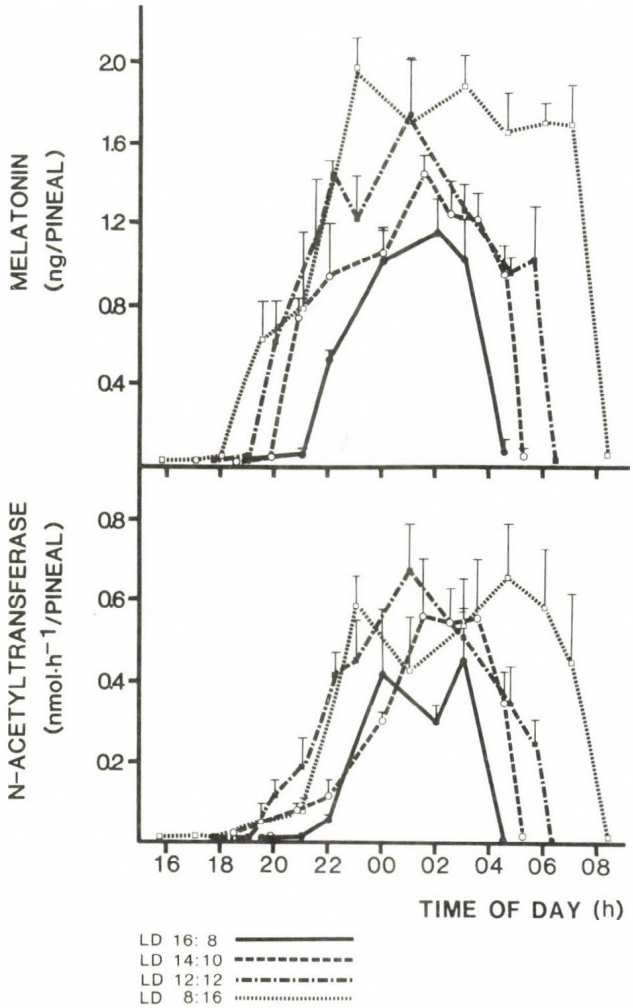

Fig. 6. Diurnal rhythm of melatonin content (above) and NAT activity (below) in adult Djungarian hamsters under different photoperiods. Photoperiods for the different curves are indicated below, dark-times were centered around midnight in every case. Animals had lived under these conditions for at least 6 weeks. Points give means (+ SEM) of 4 (light-time; 4 x 3 pineals) or 5 (dark-time; 5 x 2 pineals) determinations. Baseline values of melatonin were about 0.03 ng/pineal, of NAT activity lower than 0.01 nmol/h/pineal, i.e. at the limit of detection. Illnerová, Hoffmann and Vanecek, unpublished data.

PHOTOPERIOD	PERIOD OF HIGH MELATONIN (h)	PERIOD OF HIGH NAT ACTIVITY (h)
LD 16: 8	4.3	5.8
LD 14:10	7.5	7.5
LD 12:12	9.3	10.0
LD 8:16	10.7	10.7

Table 1. Time of high melatonin concentration ($\geq$ 0.8 ng/pineal) and of elevated NAT activity ($\geq$ 0.1 nmol/h pineal) in different photoperiods. From the data in Fig. 6.

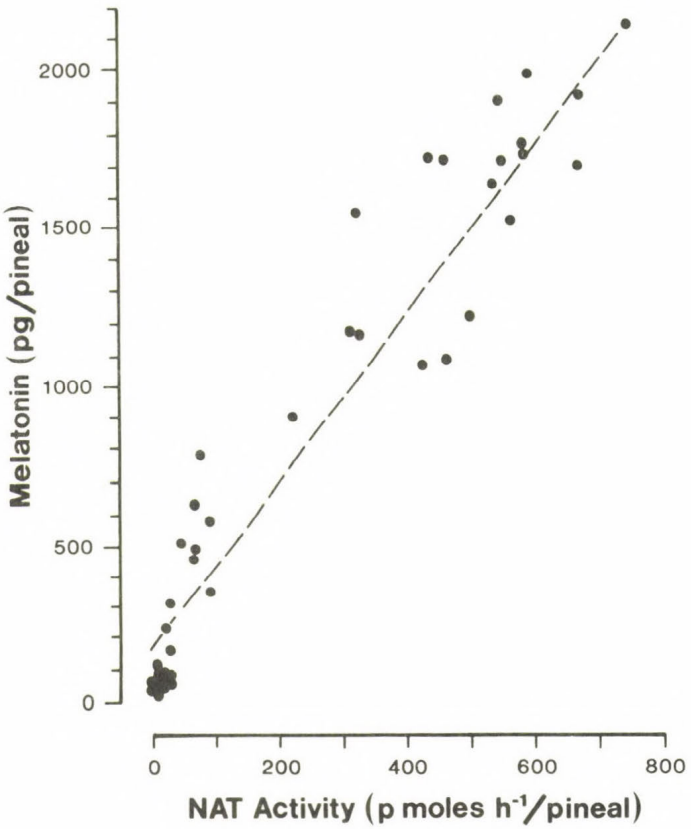

Fig. 7. Correlation between pineal NAT activity and pineal melatonin content. Each point gives the mean of 4 or 5 determinations. Values from Fig. 6, from data in Illnerová et al. 1984 and from other experiments.

gressed testes were used. Some of these animals were pinealectomized. In intact controls there was rapid recrudescence in long photoperiods, while short photoperiods prevented gonadal development. In pinealectomized animals, on the other hand, there was some modest recrudescence regardless of photoperiod, but it was significantly less than in controls in long photoperiods. Moreover, intact animals in constant light also showed only moderate recrudescence. Constant light is known to completely suppress the nocturnal rise in melatonin production and release. Thus, in general, all manipulations that prevented the nocturnal elevation of melatonin also prevented the effect of photoperiod.

These results suggest that in Phodopus the nocturnal elevation of melatonin is necessary for the transduction of photoperiodic effects, and that this process is involved in both, the inhibitory action of short photoperiods as well as the stimulatory effects of long photoperiods. In this species, the temporal pattern of pineal melatonin content and of N-acetyltransferase (NAT) activity reflect the length of the photoperiod (Fig. 6). Especially the length of time that levels are elevated above the low daytime values change with the length of the dark-time (Table 1). Since the values for both functions measured run parallel, and since there is good correlation between pineal NAT activity and melatonin content (Fig. 7), the former can also be used to determine the temporal pattern. In earlier experiments it was found that the pattern of NAT activity was indistinguishable in full long photoperiods and in skeleton photoperiods that mimicked the effect of long photoperiods on gonads and other functions (Hoffmann et al. 1981). Such findings strongly suggested that the temporal pattern of pineal melatonin synthesis and release is effective in conveying the photoperiodic message to the neuroendocrine axis. They also indicated that the duration of elevated melatonin levels might be the most important factor.

Direct evidence for this hypothesis has recently been provided by Carter and Goldman (1983a, b) in a series of elegant experiments. They used young Djungarian hamsters from a breeding colony that was derived from our stock. Some of the results are given in Fig. 8. Young males that had been maintained in long photoperiods from birth were pinealectomized and then infused daily with melatonin (Fig. 8, above). If melatonin was infused for 12 h per day, this treatment induced gonadal regression, similar to the action of short photoperiods in intact animals. However, if the same amount was infused for only 4 h a day it stimulated gonadal growth, as did long photoperiods in intact animals. In a further set of experiments, animals were reared in short photoperiods and, accordingly, had undeveloped testes (Fig. 8, below). When such hamsters were pinealectomized, infusion of melatonin for 4 h each day induced gonadal growth, while infusion for 12 h maintained the undeveloped state. Again the infusions in pinealectomized hamsters mimicked the action of photoperiod in intact animals.

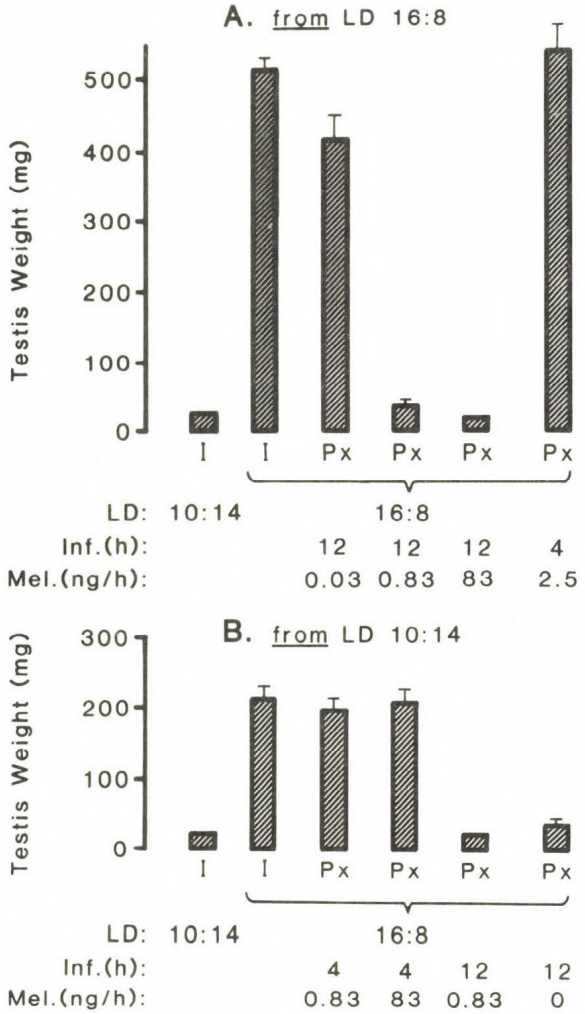

Fig. 8. Testis weight of young Phodopus after 12 days of daily infusion with melatonin. Animals were raised in long (above) or short (below) photoperiods and pinealectomized (Px) at 18 (above) or 23 (below) days of age and then exposed to the schedules indicated. Note inhibitory effect of 12 h infusion per day and stimulatory effect of 4 h infusion. I = intact controls without infusion. N = 9-12 per group. After values in Carter and Goldman (1983a, b).

In general, the e findings clearly show that the length of time high melatonin ıevels are present is the decisive signal. They also show that the same hormone, melatonin, may either induce gonadal growth or gonadal regression, depending on its temporal pattern. On the other hand, the reaction was, over a wide range,

independent of the absolute amount of melatonin application. Long times of infusion had the same effects in concentrations varying over two orders of magnitude. The same holds for short times of infusion.

Fig. 8 presents only some of the many experiments performed by Carter and Goldman. Some additional features should be mentioned. Melatonin infusions were always performed at the same time of day in these experimens, originally at night when, in intact animals, melatonin levels are high. However, when infusions were performed during the day rather than during the night, the same results ensued. This suggests that no circadian rhythm of sensitivity to melatonin seems to be involved in this mechanism.

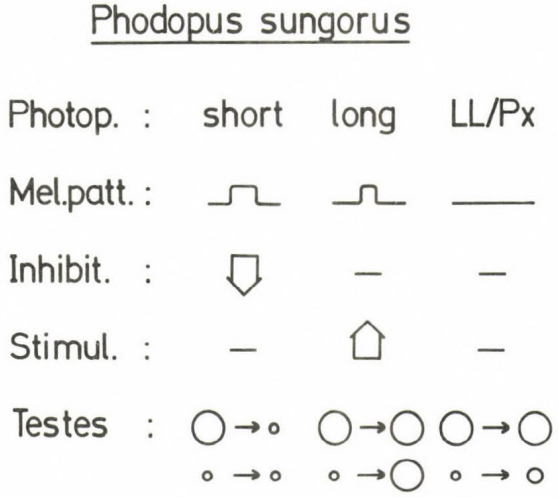

Fig. 9. Theoretical scheme for melatonin action in the photoperiodic mechanism of the Djungarian hamster. LL = continuous light, Px = pinealectomy. For further explanation see text.

If one considers the results of all the experiments mentioned here the following scheme for the pineal mechanism conveying the photoperiodic message can be constructed (Fig. 9). Short photoperiods, by the extended pattern of high melatonin levels, are inhibitory. They induce gonadal regression or maintain the regressed state (until spontaneous recrudescense sets in as occurs in animals maintained in short photoperiods for extended times = photorefractoriness). Long photoperiods, by the relatively brief nocturnal elevations of melatonin, are stimulatory, they induce fast gonadal development. If the nocturnal increase is abolished, either by pinealectomy or by exposure to continuous light, there is neither stimulation nor inhibition. In this case, large testes remain large and active, small testes show some development, but it is much less than the growth induced by long photoperiods. This scheme is certainly somewhat

oversimplified, and will have to be modified with further experimentation. At present, however, it seems to best fit the solid data on the role of the pineal gland in the photoperiodic mechanism of the Djungarian hamster. It should also be mentioned that there are indications that the same scheme also holds for mechanism of photoperiodic action on other functions like body weight and pelage color.

COMPARISON WITH OTHER SPECIES

The question arises whether the scheme for pineal function and melatonin action developed for the Djungarian hamster can be extended to other photoperiodic species. It can first be stated that in practically all photoperiodic mammals investigated in this respect, the pineal gland is involved in transducing the photoperiodic message. At least in some of them there is also evidence that pineal melatonin synthesis and release is involved in this process. However, there seem to be marked species differences in the details of the mechanism.

In sheep, there is convincing evidence that here, also, the length of time melatonin levels are elevated above the low daytime values is the effective signal from the pineal gland. An influence of extended times of high melatonin values was first suggested by Kennaway et al. (1982) and later convincingly demonstrated by Arendt et al. (1983). Recently, in a set of elegant experiments using pinealectomized ewes, and methods similar to those applied by Carter and Goldman (1983a), Bittman et al. (1983, Karsch et al. 1984) demonstrated that, in this species, the length of time of elevated melatonin values regulates initiation and termination of the sexual phase. As compared to the findings in Phodopus, the only difference is a reversal of the effects of the temporal pattern of melatonin: In sheep, long times of elevated melatonin levels are stimulatory, short times are inhibitory. Since the sheep is a shortday breeder, and since light suppresses melatonin levels, this reversal seems understandable. From evolutionary considerations these results also stress the point that melatonin is not antigonadotropic or progonadotropic per se, but that the temporal pattern of melatonin release only conveys the message from the pineal gland to the neuroendocrine axis. The details may differ in a species-specific fashion.

Two other hamster species have also been investigated in some detail, the golden hamster Mesocricetus auratus and the Turkish hamster Mesocricetus brandti. Though it has been claimed that there is no difference in the length of elevated melatonin levels in long and in short photoperiods in the golden hamster, closer inspection of published data reveals such a difference, though it is less marked than in the Djungarian hamster (see Hoffmann 1981b, Fig. 5). Thus it seems not unplausible that a mechanism similar to that shown for the Djungarian hamster applies. This is illustrated in Fig. 10 (above). As in Phodopus, short photoperiods induce gonadal regression which suggests that the corresponding melatonin pattern is inhibitory. However, while in long photoperiods there is rapid recrudescence, the

same holds if the animals are pinealectomized or if the nocturnal melatonin rise is prevented by other manipulations: Regressed testes recrudesce rapidly, as has been shown in many experiments by several laboratories (see Reiter 1981 for review). Thus, there seems to be only inhibition by the melatonin pattern typical for short photoperiods. The only effect of long photoperiods in which the pineal gland is involved is the breaking of photorefractoriness as was shown by Bittman and Zucker (1981). This is indicated by the hatched arrow in Fig. 10.

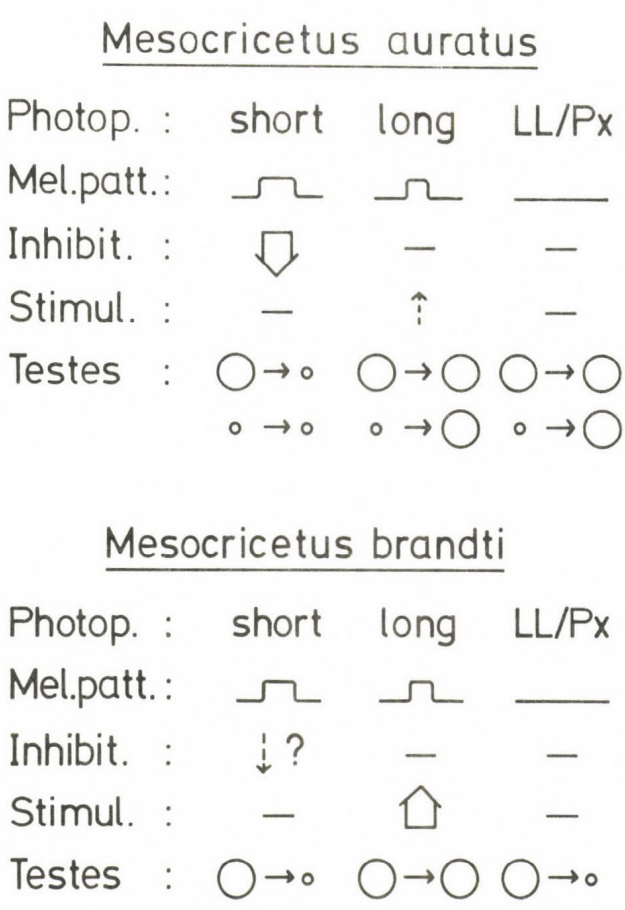

Fig. 10. Theoretical scheme for melatonin action in the photoperiodic mechanism of the golden hamster (above) and the Turkish hamster (below). For further explanation see Fig. 9 and text.

Recently, another member of the genus Mesocricetus has been investigated, the closely related Turkish hamster Mesocricetus brandti (Carter et al. 1982). The melatonin pattern in this spe-

cies was reported to be similar to that found in the golden hamster. Short photoperiods induce regression while long photoperiods maintain the active state of gonads. However, pinealectomy, constant light or other manipulations that suppress the nocturnal rise of melatonin resulted in gonadal involution. Apparently the temporal pattern of melatonin in long photoperiods is essential for stimulating the gonadal system, while its suppression and, thereby, the lack of stimulation causes gonadal collapse. In this respect the reaction of the Turkish hamster is just the opposite of that observed in the golden hamster.

It should be stressed that the scheme developed in Fig. 10 for the two Mesocricetus species is hypothetical. No patterned infusions have been performed in either species so far. However, in the golden hamster it has been shown that daily injctions of melatonin can mimic the effects of short photoperiods in long light-times if they are performed at the right time relative to the light-dark cycle in which the animals are maintained. This was first demonstrated by Tamarkin et al. (1976) and later confirmed in many other laboratories. In pinealectomized golden hamsters injections of melatonin were also effective in causing regression if they were performed three times daily in 3 h intervals, as first shown by Tamarkin et al. (1977) and later by other groups. In general, these experiments suggest that, here again, the temporal pattern of melatonin is important in conveying the photoperiodic message. No data are available for the Turkish hamster.

The golden hamster is the species in which the participation of the pineal gland in the photoperiodic mechanism was first studied, and it is certainly the species that has been most frequently investigated in this respect. The still current concept, now widely mentioned in the literature, that the pineal gland is an antigonadotropic organ, or has antigonadotropic effects, and that melatonin has antigonadotropic properties, is to a large extent based on the results of experiments using this species. In view of the findings by Carter et al. (1982) in the Turkish hamster, one might suspect that, had the Turkish hamster been studied first, and as extensively, the current concept would be that the pineal gland is a progonadotropic organ.

CONCLUDING REMARKS

The following conclusions can be drawn: In photoperiodic mammals, the pineal gland is involved in conveying the photoperiodic message. This concerns not only the gonads and gonad-influenced processes, but also other functions regulated by photoperiod. The temporal pattern of pineal melatonin synthesis and release seems to be the main factor involved in transducing the photoperiodic message, at least in some species. However, the details of this mechanism differ from species to species, even in closely related forms.

In several species there is evidence that the pineal participates, not only in conveying the inhibitory effects of photoperiod, but also the stimulatory effects. The concept that the

pineal is an antigonadotropic organ, and that melatonin is an antigonadotropic hormone, is certainly unwarranted. Such a claim, if it is based on work in photoperiodic species, has to be rejected. This does not exclude that, in non-photoperiodic processes, the pineal might have some genuine antigonadotropic effect, but this has still to be demonstrated (see also Sizonenko and Lang, this volume).

Considering these data and the resultant reformulation of our ideas concerning pineal function, and, furthermore, the dependance of these concepts on the particulars of the species under study, I think this work clearly demonstrates the importance of comparative research in physiology, a field that is currently neglected.

REFERENCES

Arendt, J., Symons, A.M., Laud, C.A., and Pryde, S.J. (1983) Melatonin can induce early onset of the breeding season in ewes. J. Endocr. 97,395-400.

Bittmann, E.L., Dempsey, R.J., and Karsch, F.J. (1983) Pineal melatonin secretion drives the reprductive response to daylength in the ewe. Endocrinology 113,2276-2283.

Bittmann, E.L., and Zucker, I. (1981) Photoperiodic termination of the hamster refractoriness: Participation of the pineal gland. Biol. Reprod. 24,568-572.

Brackmann, M., and Hoffmann, K. (1977) Pinealectomy and photoperiod influence testicular development in the Djungarian hamster. Naturwissenschaften 64,341-342.

Carter, D.S., and Goldman, B.D. (1983) Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungorus sungorus): Duration is the critical parameter. Endocrinology 113,1261-1267.

Carter, D.S., and Goldman, B.D. (1983b) Progonadal role of the pineal in the Djungarian hamster (Phodopus sungorus sungorus): Mediation by melatonin. Endocrinology 113,1268-1273.

Carter, D.S., Hall, V.D., Tamarkin, L., and Goldman, B.D. (1982) Pineal is required for testicular maintenance in the Turkish hamster (Mesocricetus brandti). Endocrinology 111,863-871.

Duncan, M.J., and Goldman, B.D. (1984) Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, Phodopus sungorus. I. Role of the gonads and the pituitary. J. Exp. Zool. 230,89-95.

Earnest, J.D., and Turek, F.W. (1984) Periodic exposure to a brief light signal stimulates neuroendocrine-gonadal activity in golden hamsters. J. Androl. 5,64-69.

Elliott, J.A. (1974) Photoperiodic regulation of testis function in the golden hamster: Relation to the circadian system. Ph. D. thesis, Univ. of Texas, Austin.

Elliott, J.A. (1976) Circadian rhythms and photoperiodic time measurement in mammals. Fed. Proc. 35,2339-2346.

Ellis, D.H., and Follett, B.K. (1983) Gonadotropin secretion and testicular function in golden hamsters exposed to skeleton photoperiods with ultrashort light pulses. Biol. Reprod. 29, 805-818.

Follett, B.K., and Robinson, J.E. (1980) Photoperiod and gonadotrophin secretion in birds. in: Reiter, R.J., and Follett, B.K. (Eds.), Progress in Reproductive Biology, Vol. V., Karger, Basel, pp. 39-61.

Gaston, S., and Menaker, M. (1967) Photoperiodic control of hamster testis. Science 158,925-928.

Hoffmann, K. (1974) Testicular involution in short photoperiodic inhibited by melatonin. Naturwissenschaften 61,364-365.

Hoffmann, K. (1977) Die Funktion des Pineals bei der Jahresperiodic der Säuger. Nova Acta Leopoldina 46,217-229.

Hoffmann, K. (1978) Photoperiodic mechanism in hamsters: the participation of the pineal gland. in: Assenmacher, I., and Farner, D.S. (Eds.), Environmental Endocrinology. Springer, Berlin-Heidelberg, pp. 94-102.

Hoffmann, K. (1979) Photoperiodic effects in the Djungarian hamster: One minute of light during darktime mimics influences of long photoperiods on testicular recrudescence, body weight and pelage colour. Experientia 35,1529-1530.

Hoffmann, K. (1981a) Photoperiodism in vertebrates. in: Aschoff, J. (Ed.), Handbook of Behavioral Neurobiology, Vol. 4, Plenum Press, New York-London, pp. 449-473.

Hoffmann, K. (1981b) Photoperiodic function of the mammalian pineal organ. in: Oksche, A., and Pevet, P. (Eds.), The Pineal Organ: Photobiology - Biochronometry - Endocronology. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 123-138.

Hoffmann, K. (1982a) The critical photoperiod in the Djungarian hamster. in: Aschoff, J., Daan, S., and Gross, G. (Eds.), Vertebrate Circadian Systems. Springer, Berlin-Heidelberg, pp. 297-304.

Hoffmann, K. (1982b) The effect of brief light pulses on the photoperiodic reaction in the Djungarian hamster Phodopus sungorus. J. Comp. Physiol. 148,529-534.

Hoffmann, K. (1984) Photoperiodic reaction in the Djungarian hamster is influenced by previous light history. Biol. Reprod. 30, Suppl. 1:55.

Hoffmann, K., and Küderling, I. (1975) Pinealectomy inhibits stimulation of testicular development by long photoperiods in a hamster (Phodupus sungorus). Experientia 31,122-123.

Hoffmann, K., and Küderling, I. (1977) Antigonadal effects of melatonin in pinealectomized Djungarian hamsters. Naturwissenschaften 64,339-340.

Illnerová, H., Hoffmann, K., and Vanecek, J. (1984) Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiods in the Djungarian hamster Phodopus sungorus. Neuroendocrinology 38,227-231.

Karsch, F.J., Bittman, E.L., Foster, D.L., Goodman, R.L., Legan, S.J., and Robinson, J.E. (1984) Neuroendocrine basis of seasonal reproduction. Rec. Progr. Hormone Res. 40,185-232.

Kennaway, D.J., Gilmore, T.A., and Seamark, R.F. (1982) Effect of melatonin feeding on serum prolactin and gonadotropin levels and the onset of estrous cyclicity in sheep. Endocrinology 110,1766-1772.

Reiter, R.J. (1981) Reproductive effects of the pineal gland and pineal indoles in the Syrian hamster and the albino rat. in: Reiter, R.J. (Ed.), The Pineal Gland, Vol. II:

Reproductive Effects. CRC Press, Boca Raton, Florida, pp. 45-81.

Tamarkin, L., Westrom, W.K., Hamill, W., and Goldman, B.D. (1976) Effect of melatonin on the reproductive system of male and female Syrian hamsters: a diurnal rhythm in sensitivity to melatonin. Endocrinology 99,1534-1541.

Tamarkin, L., Hollister, C.W., Levebvre, N.G., and Goldman, B. D. (1977) Melatonin induction of gonadal quiescence in pinealectomiced Syrian hamsters. Science 198,953-955.

PINEAL GLAND AND CENTRAL NERVOUS MECHANISMS IN THE REGULATION OF REPRODUCTION IN NON-SEASONAL BREEDERS

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

ROLE OF SEROTONERGIC MECHANISMS IN MODULATING EFFECTS OF MELATONIN UPON REPRODUCTIVE ACTIVITY IN THE RAT

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INTRODUCTION

If the large number of endocrine and non-endocrine processes the pineal organ reportedly influences are unequivocally substantiated, then the pineal would now have to be considered at least as influential as pituitary gland. Like for pituitary, the diversity of actions of the pineal would practically require that several specific active factors were synthesized and released from it. However, over past two decades, while failing to isolate different active compounds specific for pineal different effects, a wide range of studies apparently indicated that a single hormone, melatonin (aMT), has the ability to mediate all actions of pineal body, including inhibition and stimulation of the same targets. In this context, also the concept of a negative feed-back system between pineal and hypothalamo-pituitary tract, conjectured in the sixties (Fiske et al., 1962; De Gouttes,1964; Trentini et al.,1967) has become too restrictive.

As currently envisioned, the role of pineal organ appears to be more general, and presumedly that of a structure which centrally regulates endocrine and non-endocrine regulatory systems, by integrating and conveying various exogenous and endogenous stimuli, that keep the organism as a whole in proper synchrony with the external environment. However, the exact mechanism of how pineal or aMT influence physiological processes remains still obscure.

Aim of the present paper is to review studies showing that aMT is able to reproduce physiological effects of the pineal, its action being not specifically addressed to a particular function, but to a general modulation of brain activity, and to collect data supporting the proposition this effect is performed through changes of brain serotonergic system.

MELATONIN AS MEDIATOR OF PINEAL FUNCTION

Within past 15 years, substantial progress has been made in under-

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standing that pineal body functions as a neuroendocrine transducer, namely as an organ which performs the unique function of utilizing information derived from circadian, environmental changes of light and, possibly, of other phenomena (i.e. temperature and pressure) to modulate hormonal output of aMT, which provides brain with a time signal that regulates a number of neuroendocrine and neurophysiological rhythms, like locomotor activity and temperature (Binkley,1981; Redman et al.,1983), aggression and animal's emotionality (Armstrong et al.,1982), sleep organization (Sarda et al.,1983), blood pressure (Zanoboni and Zanoboni-Muciaccia, 1967), thirst regulation (Stephan and Zucker, 1972), and diuresis (Armstrong et al.,1982).

Careful consideration deserves today the controversy concerning the inhibitory or stimulatory action pineal body exerts on neuroendocrine system. Actually, it becomes increasingly clear that pineal body plays either an antigonadal role or a progonadal effect in regulation of reproductive system. A complete review of vast literature describing pineal effects on reproductive function is beyond the scope of this paper. It seems only convenient to remind briefly pertinent data of two representative groups of animals, i.e. non-seasonal and seasonal breeders.

In rat, pinealectomy (Px) results in prolongation of estrus phase of vaginal cycle (Albertazzi et al.,1966), in ovarian hypertrophy (Wurtman et al.,1959), and in increased weights of testes, seminal vescicles and ventral prostate (Motta et al.,1967), while aMT administration has contrary effects (Wurtman et al.,1963; Kappers,1962). Similar alterations are observed with exposure to continuous light (LL) for short periods (Wurtman et al.,1961), an effect reversed by aMT (Wurtman et al.,1963; Chu et al.,1964). Since pineal body mediates environmental changes mainly in lighting conditions, it is not surprising that pineal manipulations have not dramatic effects on reproduction in animals like non-seasonal breeders whose reproductive cycle is firmly regulated and stable under standard laboratory conditions. For this reason, we developed a reproducible experimental model, based on suppression of reproductive cyclicity, to demonstrate the role of pineal in rat reproductive physiology.

Rats in constant estrous anovulatory (CEA) state, induced either by anterobasal hypothalamic injuries, i.e. frontal hypothalamic deafferentation or bilateral electrolytic lesions (HI), or neonatal injection of androgen (15-250µg testosterone/rat) (NA), or exposure to LL (produced by 5 Philips TL40W-33 sources) for 120 days, were subjected to either Px or superior sympathetic cervical ganglionectomy (CGx), and/or daily s.c. administration of aMT (2 x 100µg/day).

Ablation of pineal body or depression of its synthetic activity by CGx reinstate reproductive cyclicity with repeated ovulations (Trentini et al.,1973; Mess et al.,1973) in a significant number ($P<0.01$) of animals with CEA state due to HI or NA, while Px appears ineffective in LL-CEA rats (Fig. 1). As shown in further experiments (Trentini et al.,1976), ovulation-inducing effect of Px or CGx took place through increased release of LH, though abnormally timed.

These results demonstrate that suppression of circulating pineal

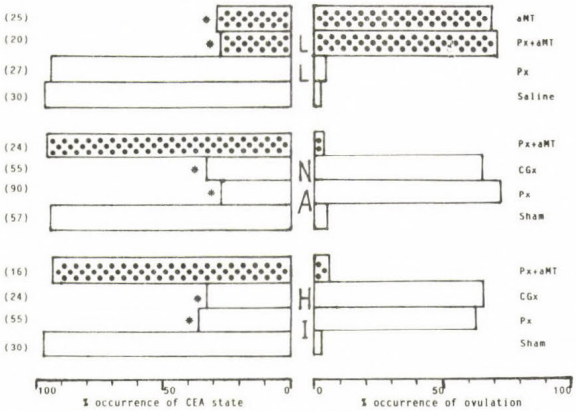

Fig.1. EFFECTS OF PINEALECTOMY (Px), SYMPATHETIC CERVICAL GANGLIONECTOMY (CGx), AND/OR aMT ON REPRODUCTIVE FUNCTION OF RATS WITH WELL-ESTABLISHED CEA STATE INDUCED BY HYPOTHALAMIC INJURY (HI), NEONATAL ANDROGENIZATION (NA), OR EXPOSURE TO CONTINUOUS LIGHT (LL).
P<0.01

secretory products removes an antigonadal factor which permits ovulation.

Accordingly, administration of aMT fully counteracts ovulation--inducing effect of either Px or CGx, indicating that aMT probably represents the pineal antigonadal factor.

On the other hand, daily administration of aMT is able to reinstate reproductive cyclicity and ovulation in rats with CEA state induced by exposure to LL, and this action is unaffected by Px (Fig. 1).

Moreover, aMT administered daily in physiological amounts from the

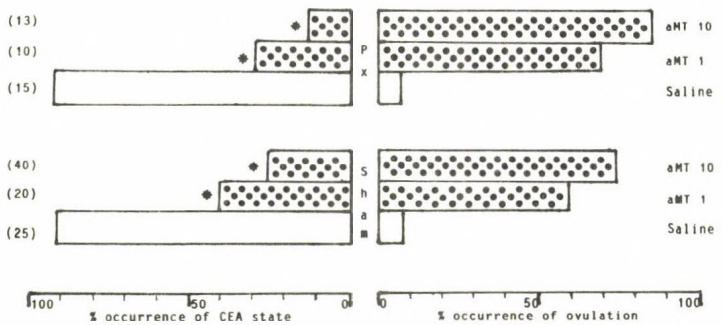

Fig.2. MAINTENANCE OF REPRODUCTIVE CYCLICITY IN SHAM- OR Px RATS EXPOSED TO LL, AND DAILY S.C. INJECTED WITH EITHER 1 OR 10 μg aMT FROM THE BEGINNING OF LL EXPOSURE.

* P<0.01

beginning of LL exposure prevents development of CEA state and maintains reproductive cyclicity (Fig. 2). Thus, aMT exerts a progonadal activity in LL conditions with an effect that is pineal-independent and mimicks the action of nyctohemeral rhythm on hypothalamo-pituitary-gonadal axis.

All together, these data demonstrate that pineal body and its main secretory product, aMT, display in rat either an antigonadal or a progonadal action under different experimental conditions.

In general, the reproductive effects of pineal body are quantitatively far greater in seasonal breeders. Restricted light exposure or chronic evening aMT injections appear to induce regression of reproductive organs in both male and female syrian hamsters (Reiter, 1980; Vaughan, 1981), as well as in djungarian hamster (Hoffmann, 1972) and several other photoperiodic mammalian species (Herbert, 1972; Pévet and Haldar-Misra, 1982). Reproductive collapse is accompanied by hormonal imbalance, with depressed plasma values of LH, FSH, prolactin and testosterone in males, while in females LH and FSH may actually be increased, although prolactin levels are usually depressed (Tamarkin et al.,1976; Reiter, 1980; Vaughan, 1981). Px completely prevents the effects of short days and aMT treatment, though twice daily injections of aMT can cause testicular regression and hormonal imbalance in Px hamsters irrespective of the photoperiod (Goldman et al., 1979). It is thus apparent that aMT injections function physiologically in the same manner as short photoperiod and may mediate the effects of short days on reproduction.

In addition to findings that indicate a strong antigonadal role for pineal and aMT, recent data indicate, as reviewed by Hoffmann at this Colloquium, that pineal is also involved in stimulation of reproductive activity. The findings of Carter et al.(1982, 1983a) suggest that in turkish hamster and in djungarian hamster aMT may be involved in both maintenance of testis function during exposure to long day photoperiod and in suppression of reproductive function in short days. By using aMT infusion of different duration, Carter et al. (1983b) demonstrated that aMT peaks of 6-h duration or less elicit a long day response, i.e. stimulation of pituitary-gonadal axis, while aMT peaks of 8- to 12-h duration elicit a short day response, i.e. inhibition of reproductive system. Other evidence suggests that aMT infusions can mimic the effect of inhibitory as well as stimulatory photoperiods also in short-day breeders (Bittman et al.,1983). Experiments of Arendt et al. (1983) provide evidence of a progonadotropic effect of aMT in ewes, by showing that aMT-treated animals were the first to initiate estrous cycle, though having aMT plasma levels higher than both short-day exposed ewes and control ewes.

As for reproductive system, pineal body seems to exert either an inhibitory or a stimulatory (Gordon et al.,1980) effect on thyroid function, though the great bulk of evidence indicates a suppressive effect (Relkin, 1978; Vaughan et al., 1982). A coincidence of counter-antigonadal and counter-antithyroid action of aMT administered via the drinking water in male golden hamster has recently been reported (Vriend and Gibbs, 1984).

Similarly, there are many indications that the pineal organ has a generalized suppressive influence on growth hormone secretion (Smythe and Lazarus, 1974a), and on glucocorticoid secretion (Wurtman et al.,1961; Vaughan et al., 1972), but findings from different laboratories are contradictory. In man, prior administration of aMT blocks the rise in plasma GH levels, induced by either exercise or hypoglicemia (Smythe and Lazarus, 1974a), but aMT alone is associated with a rise in plasma GH levels (Smythe and Lazarus, 1974b). In the rat, Px has been shown to

significantly decrease serum GH values in light, but not in dark (Ronnekleiv and McCann, 1978), and to disrupt entrainment of GH rhythm (Willoughby et al., 1978). With regard to adrenocortical function only borderline modifications or no changes have been reported following Px in stress response and circadian adrenocortical rhythm (Klein et al., 1979).

All reported data show that pineal organ influences as many biochemical, physiological, endocrinological and behavioral processes as probably no other organ in the body. Furthermore, they demonstrate that aMT is the hormonal product that reproduces, and probably mediates all actions of the pineal, as far as the seemingly paradoxical activity of inhibiting or stimulating same physiological processes.

Accordingly, though in last few years several indole compounds have been recovered from pineal tissue (for survey, see: Balemans, 1979; Smith, 1983), none has shown to produce the dramatic reproductive effects that aMT does, their physiological role being far from clarification.

In a work directed to investigate whether other pineal indoles possess an activity similar to that of aMT in preventing the effect of LL on rat ovarian cyclicity (Trentini et al., 1982), we demonstrated that all indoles tested are significantly less active than aMT in preventing the development of CEA state in LL rats. Particularly, 5-methoxytryptophan and 5-methoxyindole-3-acetic acid failed to show any activity, whereas N-acetylserotonin (aHT), 5-hydroxytryptophol (HL), 5-methoxytryptophol (ML) and 5-methoxytryptamine (MT) maintained ovulation in about 30% of LL rats (Fig. 3).

Similar results were obtained by Vaughan et al. (1972), Sackman et al. (1977) and Reiter (1980) by studying the gonad-inhibiting activity of several pineal indoles in male hamsters. All indoles tested did not shared aMT's ability to suppress reproductive physiology, though some significant effects were presented by aHT, ML, HL and MT, as shown at this Colloquium by Pévet. By testing the potential antigonadotropic properties of a large number of natural and synthetic analogues of aMT, Richardson et al. (1983) show that 6-chloromelatonin (aMT6Cl) is the only compound as effective as aMT in inhibiting the hamster reproductive axis. When acutely administered to rats, aMT6Cl appears to be a more potent inhibitor of ovulation than aMT (Clemens et al., 1980), its enhanced activity being purportedly referred to its increased plasma half-life due to halogenation, compared to aMT (Frohn et al., 1980). However, by using the same compounds including aMT6Cl, this same group (Vaughan et al., 1983) failed to detect any effect on circulating T4 levels, which were instead significantly depressed by injection of aMT, in our opinion questioning the suggested aMT-agonist effect of aMT6Cl.

By investigating the effect of aMT and other indole derivatives in maintaining ovulation in rats kept in LL, we evaluated also the possible influence they exert on HIOMT activity of pineal body in the 24-h period (Trentini et al., 1983). All indoles tested appear to stimulate significantly HIOMT activity for the synthesis of aMT inhibited by exposure to LL, in some way reactivating at a low level daily rhythm of aMT production. Only administration of aMT does not influence its own synthesis (Fig. 4).

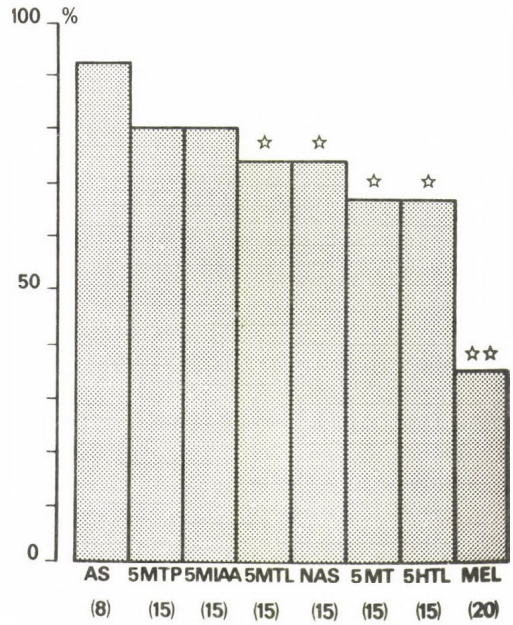

Fig. 3. % occurrence of CEA state in rats exposed to LL and injected daily with 10ug of different pineal indoles.
As= Alcoholic saline
5MTP= 5-methoxytryptophan
5MIAA= 5-methoxy-3-indole acetic acid
5MTL= 5-methoxytryptophol
NAS= N-acetylserotonin
5MT= 5-methoxytryptamine
5HTL= 5-hydroxytryptophol
MEL= melatonin

On the basis of these results, the effect that indole derivatives other than aMT exert on reproductive function seems to be at least partly mediated through aMT.

Though these results need to be confirmed by demonstrating parallel changes in aMT serum levels, they appear to suggest that the whole metabolic machinery of pineal body is addressed to the synthesis of aMT, once again indicated as the main or, probably, only pineal hormonal product.

Considering the multitude of functions pineal body affects via the secretion of a single product it is unlikely it possesses so many specific activities, therefore its action must take place at a higher general level of integration.

Based on studies in rodents and monkey, suprachiasmatic nucleus (SCN) of hypothalamus, rather than pineal, has been recognized to play a key role in organization of circadian rhythms of behavioral and endocrine functions (Klein, 1978; Moore, 1978; Williams, 1983). Destruction of SCN abolishes circadian rhythm of plasma corticosterone (Moore and Eichler, 1972), as well as rhythms of drinking and locomotor activity (Stephan and Zucker, 1972), sleep-wake cycle (Menaker et al., 1978), and eating-fasting cycle (Nagai et al., 1978). Ample evidence also suggests that SCN is responsible for synchronization of gonadal activity with environmental light, without involving pineal organ (Meyer and Quay, 1976).

Since SCN has been shown to contain a large number of aminergic nerve fibers, originating from different neurotransmitter systems, particularly from midbrain raphe nuclei (Fuxe and Jonsson, 1974; Herbert and Yates, 1976, Steinbusch, 1981), we investigated the possible involvment of these structures in the regulation of rat ovarian cycle. By means of electro-

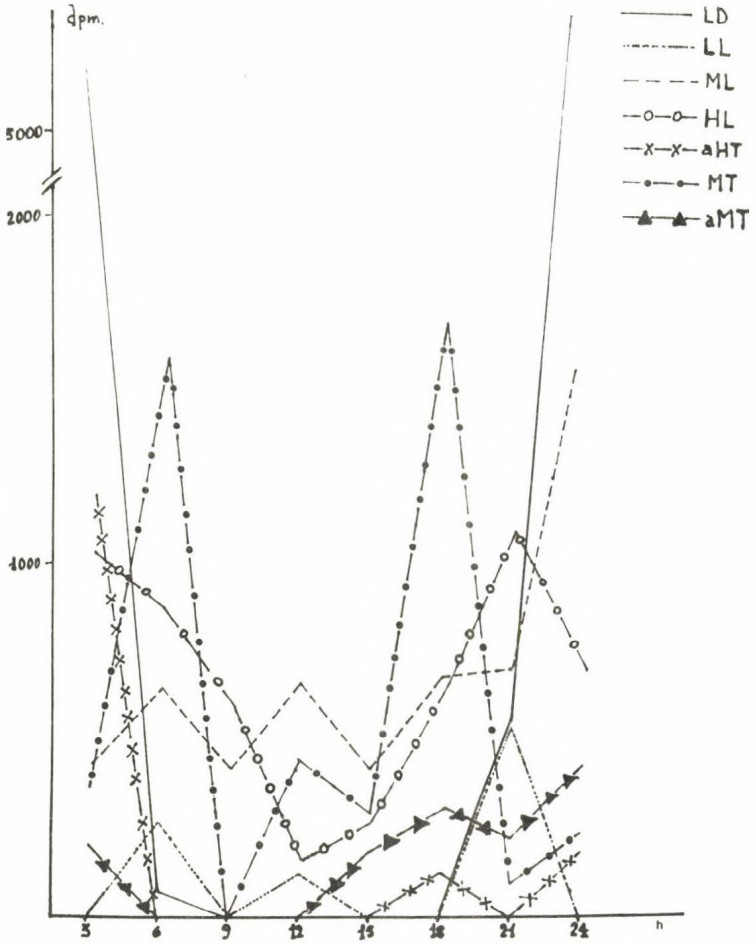

Fig.4. 24 h VARIATIONS IN PINEAL HIOMT FOR THE SYNTHESIS OF aMT IN LL-CEA RATS TREATED DAILY WITH DIFFERENT INDOLE DERIVATIVES.

lytic destruction or of specific neurotoxic lesions (by using: a. 5,7-dihydroxytryptamine (5,7-DHT) and desmethylimipramine (DMI) for serotonergic nerve endings; b. 6-hydroxydopamine (6-OHDA) and DMI for dopaminergic (DA) nerve endings; and c. 6-OHDA and benztropine (BT) for norepinephrinergic (NE) nerve endings) of SCN or of midbrain dorsal (DR) and median (MR) raphe nuclei placed by stereotaxic technique, we demonstrated that electrolytic destruction of SCN induces CEA state in more than 90% of rats maintained in normal LD cycle, while lesions of midbrain raphe nuclei do not affect reproductive cyclicicty. Among rats bearing different neurotoxic lesions, those with damage of HT and NE nerve terminals of SCN presented a significant prolongation of estrus phases, sometimes resulting in CEA state that reaches significance in HT-lesioned rats.

Present results confirm previous data and show that the absolute

anatomical integrity of SCN is only essential among the structures investigated for maintenance of rat reproductive cyclicity . They demonstrate also that serotonergic component of SCN plays an important role in this mechanism.

Because SCN has been implicated in controlling not only circadian rhythms and ovulatory cycle, but also pineal N-acetyltransferase activity and aMT secretory rhythm (Moore and KLein, 1974; Klein, 1978), and conversely aMT has been identified in SCN, where it reaches peak values during the dark phase (Bubenik et al., 1976; Cassone et al., 1983), as previously suggested at ninth Symposium on Comparative Endocrinology (Trentini et al., in press), it seems correct to regard pineal body as an inferior clock mechanism driven by SCN, which in turn dampens the activity of the driving oscillator by means of aMT.

Table 1. PERCENT OCCURRENCE OF CEA STATE IN RATS BEARING ELECTROLYTIC DESTRUCTION OR NEUROTOXIC LESION OF SUPRACHIASMATIC NUCLEUS (SCN), OR MEDIAN (MR) OR DORSAL (DR) RAPHE NUCLEI.

Type of lesion	S C N		M R		D R	
	No/total	%	No/total	%	No/total	%
1. Sham	1/15	6.6	0/13	0.0	0/12	0.0
2. Electrolytic	10/11	91.9**	0/10	0.0	0/16	0.0
3. 5,7-DHT	6/14	42.9*	2/12	16.7	1/10	10.0
4. 6-OHDA + BT	3/8	37.5	2/10	20.0	2/10	20.0
5. 6-OHDA + DMI	1/12	8.3	1/10	10.0	1/12	8.3

5,7-DHT= 5,7-dihydroxytryptamine; 6-OHDA= 6-hydroxydopamine;
BT= Benztropin; DMI= Desmethylimipramine
* $P<0.05$ ** $P<0.01$

POSSIBLE MECHANISM OF ACTION OF MELATONIN

Accepted that, at least in seasonal breeders, the duration of daily exposure to aMT is the element by which changes in day-length are transmitted to different physiological systems (Hoffmann, 1981; Bittman et al., 1983; Carter and Goldman, 1983b), and that aMT probably acts through specific cell receptors (Niles et al., 1979; Cardinali et al., 1979; Vacas and Cardinali, 1979), biochemical mechanisms by which aMT exerts its activity still require clarification.

A critical examination of Literature reveals that HT brain system regulates same physiological, endocrinological and behavioral processes previously reported to be affected by pineal body and aMT (for survey, see: Morimoto and Yamamura, 1979; Kordon et al., 1981; Fornal and Radulovacki, 1983; Pucilowski and Kostowski, 1983).

Several years ago, we suggested a possible role of brain HT system in the ovulation-inducing effect of Px in rats with CEA state induced by HI, by demonstrating that the effect of Px, previously shown to be suppressed

by aMT, was abolished by HT administration. The tentative conclusion was that the decrease of aMT levels consequent on Px should have depressed brain HT concentration, thus permitting ovulatory release of LH (Tima et al., 1973). This hypothesis was further supported by the demonstration that simple decrease of brain HT levels induced either by injection of pCPA or by feeding animals with a tryptophan-poor (W-p) diet provokes ovulation in a significant number of HI-CEA rats (Table 2) (Trentini et al., 1974).

Table 2. EFFECTS OF MANIPULATIONS OF BRAIN HT SYSTEM ON THE OVULATION-INDUCING EFFECT OF Px OR aMT TREATMENT IN RATS WITH CEA STATE INDUCED EITHER BY HYPOTHALAMIC INJURY (HI) OR EXPOSURE TO LL.

Experimental Groups	occurrence of ovulation		Statistical significance
	No/total	%	
HI			
1. Sham	0/32	0.0	
2. Px	11/17	64.7	$P<0.01$ vs.1
3. Px + HT	2/25	8.0	$P<0.01$ vs.2
4. pCPA	28/52	53.8	$P<0.05$ vs.1
5. W-p	11/30	36.6	$P<0.05$ vs.1
LL			
6. Saline	0/17	0.0	
7. aMT (2x100ug)	18/25	72.0	$P<0.01$ vs.6
8. aMT + Meth.	6/20	30.0	$P<0.01$ vs.7
9. aMT + W-p	7/25	28.0	$P<0.01$ vs.7
10. aMT + W-p + HW	5/20	75.0	$P<0.01$ vs.9

Meth= Methiothepin 2x10mg/kg; W-p= Tryptophan-poor diet; HW= 5-hydroxytryptophan.

On the basis of these results, we hypothesized that experimental manipulations of brain HT should also affect the ovulation-inducing effect of aMT in rats with CEA state induced by exposure to LL. In this experimental condition, either administration of Methiothepin, a HT receptor antagonist or the W-p diet significantly blocked the ovulatory effect of

aMT. Daily injection of HT precursor 5-hydroxytryptophan (HW) was sufficient to re-establish the ovulation-inducing effect of aMT, suppressed by W-p diet (Table 2) (Trentini et al., 1978,1979). The same experimental manipulations of brain HT also resulted to block the re-appearance of ovulatory cyclicity in LL-CEA rats replaced in normal LD cycles (Table 3) (Mess et al., 1981). As today generally accepted, the conclusion was that daily administration of aMT reproduces the effects of the dark phase of nyctohemeral rhythm, and that HT system is involved in mediating these effects on reproductive function.

All together, these experiments demonstrate that brain HT, like aMT, exerts either a stimulatory effect or an inhibitory role on ovulation. Modern studies agree in recognizing this dual effect of brain HT on reproductive function (Coen and MacKinnon, 1979; Héry et al., 1982; Walker, 1982,1983) .

In an attempt to explain these apparently conflicting results, we developed a working hypothesis according to which a "threshold or critical level" of HT is required to elicit or permit pre-ovulatory surge of LH (Trentini et al., 1974; Mess et al., 1979, 1984). Actually, it has been firmly established that HT plays a facilitatory effect on phasic release of LH, when regulated within certain limits of synthesis and degradation that produce regular changes in its hypothalamic content. Therefore, previous concept of "critical level" has to be integrated with the concept of "critical period" (Coen and MacKinnon, 1979; Héry et al., 1982), to mean that HT's influence upon LH surge, like LH surge itself, is linked in some way to the time of the day. Drugs, like HW or pCPA, which enhance or depress HT synthesis, block reproductive function in normal female rats (Héry et al., 1976), displaying the gonad-inhibitory effect of HT, because they abolish or disrupt daily fluctuations in hypothalamic HT content, like steady-state changes in lighting conditions (Wurtman et al., 1961; Chu et al., 1964; Watts and Fink, 1981).

In our experiments, brain HT manipulations elicit ovulation, because they modify (perhaps by inducing a sort of fluctuation) brain HT content of animals which are sensitized to LH facilitatory effect of HT by steady-state plasma estradiol levels of CEA state. Similar results have been obtained by Walker (1982a) by reinstating LH surges in aging CEA rats by administration of HT neuroleptics. Therefore, HT circadian rhythm regulates ovulatory cycle by providing an intermittent daily signal, occurring during the time of maximal hypothalamic HT turnover, which facilitates estrogen positive feedback on ovulatory surge of LH (Héry et al., 1982; Walker, 1982, 1983)

The same phasic trigger mechanism has been implicated in secretion of several other hormones, as ACTH, TSH, GH and prolactin (Szafarczyk et al., 1980; Kordon, 1981; Héry et al., 1982; Smythe et al., 1982).

On the other hand, brain HT activity varies daily and throughout the year with a pattern that correlates with rhythm of lighting conditions and of pineal activity (Binkley et al., 1977; Valzelli, 1977). Px reduces hypothalamic content of HT and HA (Moszkowska et al., 1971; Sugden and Morris, 1979), increases monoamine oxidase activity (Urry and Ellis, 1972;

Table 3. OVULATION PROVOKED BY REPLACEMENT OF LL-CEA RATS INTO CYCLIC LD (14:10) ENVIRONMENT, AND COUNTER-ACTION OF THIS EFFECT BY BRAIN HT MANIPULATIONS.

Experimental Groups	Occurrence of ovulation		Statistical significance
	No/total	%	
1. LL	0/17	0.0	
2. LD	30/45	66.6	$P<0.01$ vs.1
3. LD + Meth	2/19	10.5	$P<0.01$ vs.2
4. LD + W-p	12/25	48.0	$P<0.05$ vs.2
5. LD + W-p + HW	10/10	100.0	$P<0.01$ vs.4

Meth= Methiothepin 2x10mg/kg; W-p=Tryptophan-poor diet
HW= 5-hydroxytryptophan 1mg/kg.

Olcese and de Vlaming, 1979), and alters circadian pattern of HT activity rhythm (Sugden and Morris, 1979; Healy et al., 1981; Olcese et al., 1981; Cassone et al., 1983), as well as HT uptake by hypothalamic synaptosomes (Cardinali et al., 1975). However contrary results have been also reported by Niles et al. (1983) and Steger et al. (1984). In these experiments either hypothalamic HT content has been evaluated instead of its activity rhythm (Niles et al., 1983), or the effect of Px has been investigated in hamsters kept in short photoperiods (Steger et al., 1984). Same lighting condition appears to mask HT-influencing action of Px in the goldfish, a species in which pineal removal provokes instead significant daily changes of HT in long photoperiods (Olcese et al., 1981). Perhaps the exposure of seasonal breeders to long photoperiods would result a more suitable model to demonstrate the effect of Px on brain HT activity, bearing in mind afore-mentioned data indicanting that pineal body stimulates reproductive function in long photoperiods. Accordingly, a substantial amount of evidence has accrued favoring the notion that aMT affects HT activity of hypothalamus and midbrain (Antòn-Tay, 1968; Cotzias et al.,1971; Cardinali et al., 1975; Yates and Herbert, 1976,1979; Olcese et al., 1981).

By showing that aMT, like Methiothepin, increases hypothalamic HT content and terminates LH surge in proestrous rat when administered at 19.00, Walker (1982b) suggests that aMT secretion provides a definitive end-point for LH surge, when appropriately timed. But, as previously shown in our experiments, Methiothepin, as W-p diet , blocks the ovulatory effect of aMT, when injected twice daily at 8.00 and 18.00 in LL-CEA rats, demonstrating that the function of pineal body and aMT on reproduction is somewhat more complex than pure termination of LH surge. Accordingly, circadian rhythm of hypothalamic HT activity alters after Px, and LH surge "drifts" from within the characteristic time limits (Trentini et al., 1976; Walker, 1982b).

The demonstration that Px and/or aMT injection affect rhythm of hypo-

thalamic HA content, but not that of HT concentration, leads Cassone et al. (1983) to the tentative conclusion that the rhythm of pineal aMT modulates turnover and/or release of hypothalamic HT, while a different oscillator, possibly SCN, generates rhythm of HT content. Our preliminary data in LL-CEA rats appear to support this conclusion (Table 4)(De Gaetani et al., 1980). In LL, midbrain HT and HA contents are significantly lower than those of LD controls, without apparent modification in ratio of HA to HT. On the other hand, LL does not modify HT and HA concentration in medial basal hypothalamus, where instead it appears to significantly depress their ratio. Because the ratio of concentration of HA to HT provides an effective index of turnover rate and neuronal activity of HT (Smythe et al., 1982), exposure to LL appears to decrease HT neural activity of medial basal hypothalamus , but not of midbrain , where it only provokes a significant decrease in HT content. Daily administration of aMT, like replacement into LD cycles, together with reinstatement of ovulation induces a significant rise in midbrain content of HT and HA, that reach values similar to those of LD controls, but, again, do not influence the ratio of HA to HT. The reverse effect is observed in medial basal hypothalamus, where HA/HT ratio is only increased to a level even higher than that of LD controls. No variations were detected in HT cerebral cortex with any type of experimental treatment.

Therefore, aMT and LD cycle appear to exert similar effects on midbrain and hypothalamic HT system.

From these preliminary experiments, keeping in view the limitation consequent on the single-time point measurement we did, though performed during the time of greater physiologic HT turnover (Héry et al., 1982; Walker, 1983), the tentative conclusion can be drawn that pineal gland and

Table 4. CHANGES IN HT CONCENTRATION AND METABOLISM OF BASAL HYPOTHALAMUS AND MIDBRAIN OF LL-CEA RATS OVULATING AFTER aMT TREATMENT (100µg/day) OR REPLACEMENT IN NORMAL LD CYCLES.

Treatment	N.	Basal hypothalamus			Midbrain		
		H T	H A	HA/HT	H T	H/A	HA/HT
LL	23	5.05±0.90	1.45±0.31	0.28±0.08	1.85±0.14	0.97±0.15	0.52±0.02
LL + aMT	10	2.66±0.45	1.24±0.42	0.46±0.02[b]	2.54±0.20[a]	1.28±0.13	0.50±0.04
LL + LD	11	4.47±0.94	2.29±0.84	0.51±0.09[b]	3.19±0.69[a]	1.90±0.39[b]	0.60±0.06
LD	10	5.50±0.52	1.97±0.46	0.36±0.03[a]	2.88±0.29[b]	1.62±0.17[a]	0.56±0.04

a = $P<0.05$ b = $P<0.01$ vs. LL

aMT regulates reproduction and brain HT system probably by stimulating midbrain HT synthesis and modulating hypothalamic HT rhythmic activity.

With the purpose of finding further support to this conclusion, we undertook a new series of experiments, addressed to evaluate the ability of aMT to prevent the development of CEA state when administered daily from the beginning of LL exposure to rats bearing lesions of SCN or of midbrain raphe nuclei. Rats were subjected to either electrolytic destruction or selective neurotoxic lesion of SCN, DR or MR. The neurotoxic lesion, induced by microinjection of 5,7-DHT in rats pretreated with DMI to prevent destruction of noradrenergic system, was performed with the specific aim of evaluating the possible role of non-serotonergic neurotransmitters in ovulation-maintaining effect of aMT. As previously reported, electrolytic destruction of SCN provokes CEA state in 92% of animals in normal LD conditions, with an incidence significantly higher than that induced by neurotoxic lesion ($P<0.05$). Neither electrolytic, nor pharmacological damage of DR and MR affects ovulation in LD conditions. On the other hand, not any lesion appears to hinder the development of CEA state in rats kept in LL. With regard to ovulation-maintaining effect of aMT, both types of lesions appear highly inhibitory when placed in SCN. Instead lesions of midbrain raphe nuclei, and particularly of MR, are only effective when electrolytic destruction is concerned. Neurotoxic damage of

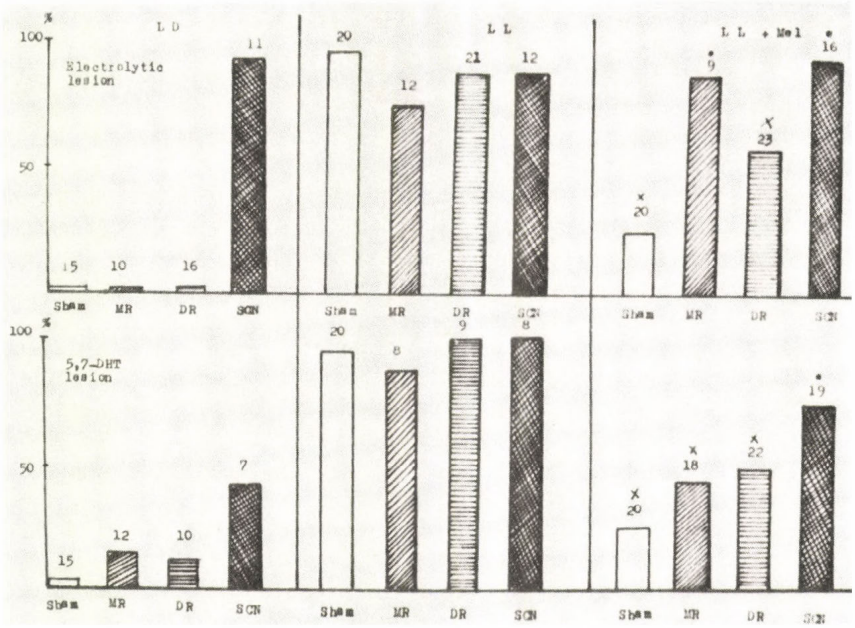

Fig. 5. Percent occurrence of CEA state in rats maintained in normal LD cycles or in LL and treated daily with 10µg Mel (aMT), and bearing either electrolytic destruction or 5,7-DHT lesion of SCN or of midbrain dorsal (DR) or median (MR) raphe nucleus. • $P<0.01$ vs sham LL+Mel; ✘ $P<0.01$ vs corresponding LL and LD.

serotonergic components of both nuclei results in a not-significant reduction of the ovulation-maintaining effect of aMT (Fig. 5) (Rúszás et al.,1982).

All together the results of these experiments confirm that the absolute integrity of SCN, and particularly of its HT component, is only essential among the structures investigated to regulate ovulatory cyclicity and to mediate reproductive modulatory effect of aMT. Glass and Lynch (1982) reach similar conclusion, by showing that intrahypothalamic injections of aMT only elicit gonadal regression, when located into vicinity of SCN.

Our results also demonstrate that midbrain raphe nuclei play a minor role, probably a side effect, in this mechanism.

In this connection, the significant prevention of aMT action consequent on midbrain electrolytic destruction, but not on specific neurotoxic damage, seems to suggest that destruction of non-serotonergic pathways located in the same areas could contribute to the effect of electrolytic lesions. Accordingly, we showed that neurotoxic lesion of SCN norepinephrinergic system affects ovarian function of rats in normal LD cycles, and Niles et al. (1983) and Steger et al. (1984) reported that short photoperiods and Px influence cortical and hypothalamic catecholamine levels and turnover.

These data, which suggest a possible involvment of other neurotransmitter systems in this neuroendocrine process, will be further discussed by Dr. Rúzsás.

Still a final point that deserves consideration is the possible functional meaning of aMT-induced increase in midbrain HT content, as demonstrated also by several previous reports (Antòn-Tay et al.,1961; Cotzias et al.,1971; Cassone et al.,1983).

Midbrain is known as the major source of HT projections to forebrain and SCN, whose metabolic activity is inhibited by these HT afferences at particular time of the day in relation with phasic release of LH (Héry et al., 1982). However, our results suggest that ascending HT system is not essential for aMT-induced ovulatory surge of LH.

Accordingly, attempts to lesion midbrain raphe nuclei failed to inhibit daily fluctuations of several hormones, including LH, while they resulted in a lower overall mean serum level of the same hormones (Kordon et al.,1981).

Thus, the possibility exists that aMT may influence the amplitude of cyclic LH release trough midbrain HT system.

On the other hand, aMT action on a primary source of HT fibers to forebrain may have consequences for more generalized changes in overall brain excitability, involving neurophysiological and behavioral processes (Healy et al., 1981; Pucilowski and Kostowsky, 1983).

CONCLUDING REMARKS

1. Pineal organ either inhibits or stimulates a variety of neurophysiological and neuroendocrine processes.

2. aMT appears to reproduce all activities of the pineal.

3. Other indole derivatives are either ineffective or less effective than aMT. One of their possible functions, granted that others exist, is to stimulate pineal HIOMT activity for aMT synthesis.

4. Reasonably, aMT cannot specifically influence so large a variety of specific functions. Its action has to perform at a high general level of integration.

5. Brain HT system is a good candidate for aMT action; it regulates same functions aMT affects.

6. aMT influences HT content and/or turnover of hypothalamus and midbrain. Absolute integrity of SCN and its HT component is essential to mediate aMT action on reproduction.

7. aMT seems to modulate neuroendocrine and neurophysiological processes via hypothalamic and mesencephalic serotonergic system.

REFERENCES

Albertazzi E.,Barbanti Silva C.,Trentini G.P. and Botticelli A.(1966): Influence de l'épiphysectomie et du traitement avec la 5-hydroxytryptamine sur le cycle oestral de la ratte albinos. Ann. Endocr. 27,93-100.

Antòn-Tay F., Chou C., Antòn S., and Wurtman R.J.(1968): Brain serotonin concentration: elevation following intraperitoneal administration of melatonin. Science 162,277-278.

Arendt J., Symons A.M., Land C.A. and Pryde S.J.(1983): Melatonin can induce early onset of the breeding season in ewes. J. Endocr. 97,395-400.

Armstrong S.,Ng K.T.and Coleman G.J.(1982):Influence of the pineal gland on brain-behavior relationships.In The pineal gland:extra-reproductive effects, Reiter R.J.(Ed), CRC Press,Boca Raton,Fla,pp.81-106.

Balemans M.G.M.(1979): The indole metabolism in the pineal gland. Prog. Brain Res. 52,222-229.

Binkley S.(1981): Pineal biochemistry: comparative aspects and circadian rhythms.In The pineal gland:anatomy and biochemistry, Reiter R.J.(Ed.), CRC Press,Boca Raton,Fla,pp.155-172.

Binkley S.,Riebman J.B. and Reilly K.B.(1977): Time keeping by the pineal gland. Science 197,1181-1183.

Bittman E.L., Dempsey R.J. and Karsch F.J. (1983): Pineal melatonin secretion drives the reproductive response to daylength in the ewe. Endocrinology 113,2267-2283.

Bubenik G.A.,Brown G.M. and Grota L.J.(1976): Differential localization of N-acetylated indolealkylamines in CNS and Harderian gland using immunohistochemistry. Brain Res. 118,417-427.

Cardinali D.P.(1975): Changes in hypothalamic neurotransmitter uptake following pinealectomy, superior cervical ganglionectomy or melatonin administration to rat. Neuroendocrinology 19,91-95.

Cardinali D.P.,Nagle C.A.,Freire F. and Rosner J.M.(1975): Effects of melatonin on neurotransmitter uptake and release by synaptosome-rich homogenates of the rat hypothalamus. Neuroendocrinology 18,72-85.

Cardinali D.P.,Vacas M.I. and Boyer E.E.(1979): Specific binding of mela

tonin in bovine brain. Endocrinology 105,437-441.
Carter D.S.and Goldman B.D.(1983a): Progonadal role of the pineal in the djungarian hamster (Phodopus sungorus sungorus): mediation by melatonin. Endocrinology 113,1268-1273.
Carter D.S.and Goldman B.D.(1983b):Antigonadal effects of timed melatonin infusion in pinealectomized male djungarian hamsters(Phodopus sungorus sungorus):duration is the critical parameter. Endocrinology 113,1261-1267.
Carter D.S., Hall V.D., Tamarkin L. and Goldman B.D.(1982): Pineal is required for testicular maintenance in the Turkish Hamster (Mesocricetus brandti). Endocrinology 111,863-871.
Cassone V.M., Lane R.F. and Menaker M.(1983): Daily rhythms of melatonin metabolism in the medial hypothalamus of the chicken: effects of pinealectomy and exogenous melatonin. Brain Res. 289,129-134.
Chu S.W., Wurtman R.J. and Axelrod J.(1964): An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology 75,238-242.
Clemens J.A., Flaugh M.E., Parli J. and Sawyer B.D.(1980): Inhibition of luteinizing hormone release and ovulation by 6-chloro- and 6-fluoromelatonin. Neuroendocrinology 30,83-87.
Coen C.W. and MacKinnon P.C.B.(1979): Serotonin involvement in the control of phasic luteinizing hormone release in the rat: evidence for a critical period. J. Endocr. 82,105-113.
Cotzias G.C.,Tang L.C.,Miller S.T.T. and Ginos J.Z.(1971): Melatonin and abnormal movements induced by L-dopa in mice. Science 173,450-452.
De Gaetani C., Poggioli R., Ferrari P., Mess B. and Trentini G.P.(1980): Is the role of melatonin in induction of ovulation in the light-induced constant estrous anovulatory state mediated through the brain serotonergic system? Reprod.Nutr.Dévelop. 20,1893-1898.
De Gouttes N.(1964): Etude de quelques effets de la castration pratiquée à la naissance chez le rat male. C. R. Soc.Biol.158,1260-1267.
Fiske V.M., Pound J. and Putnam J.(1962): Effect of light on the weight of the pineal organ in hypophisectomized, gonadectomized, adrenalectomized or thiouracil-fed rats. Endocrinology 71,130-133.
Fornal C. and Radulovacki M.(1983): Sleep suppressent action of fenfluramine in rats.I. Relation to postsynaptic serotonergic stimulation. J.Pharmacol.exptl.Ther. 225,667-674.
Frohn M.A.,Smith I. and Finnie M.D.A.(1980): Mass spectrometric and chromatographic characteristics of neutral and acid 5-methoxyindoles and some related compounds. Biomed. Mass Spectrometry 7,294-300.
Fuxe K.and Jonsson G.(1974): Futher mapping of central 5-hydroxytryptamine neurons studied with neurotoxic dihydroxytryptamine. Adv.Biochem. Psychopharm. 10,1-12.
Glass J.D. and Lynch G.R.(1982): Diurnal rhythm of response to chronic intrahypothalamic melatonin injections in the white-footed mouse, Peromyscus leucopus. Neuroendocrinology 35,117-122.
Goldman B., Hall V., Hollister C., Roychoudbury P., Tamarkin L. and Westrom W.(1979): Effects of melatonin on the reproductive system in intact and pinealectomized male hamsters maintained under various photoperiods. Endocrinology 104,82-88.

Gordon J., Morley J.E. and Hershman J.M.(1980) :Melatonin and thyroid. Horm.Metab.Res. 12,71-73.

Healy M.D., Perez J.A., Rivera A., Limon D., Torrez R., Moreno G., Stavinoha W.B. and Modak A.T.(1981): Circadian rhythmicity of motor activity and serotonin levels in the brains of intact and pinealectomized rats in magnesium deficiency.In Chronopharmacology and Chronotherapeutics, Walker C.A., Soliman K.F.A. and Winget C.M.(Eds.), Florida Univ.Found., Tallahassee ,Fla,pp.67-75.

Héry M., La Plante E., Pattou E. and Kordon C.(1976): Participation of serotonin in the phasic release of LH. I. Evidence from pharmacological experiments. Endocrinology 99,496-503.

Héry M., Faudon M., Dusticier G. and Héry F.(1982): Daily variations in serotonin metabolism in the suprachiasmatic nucleus of the rat: influence of oestradiol impregnation. J.Endocr. 94,157-166.

Hoffmann K.(1972): Melatonin inhibits photoperiodically induced testis development in a dwarf hamster. Naturwissenschaften 59,218-219.

Hoffmann K.(1981): The role of the pineal gland in the photoperiodic control of seasonal cycles in hamsters.In Biological clocks in seasonal reproductive cycles, Follett B.K.and Follett D.E.(Eds.), Wright, Bristol, pp.237-250.

Kappers J.Ariens(1962): Melatonin, a pineal compound.Preliminary investigations on its function in the rat. Gen.comp.Endocrinol. 2,610-611.

Klein D.C.(1978): The pineal gland: a model of neuroendocrine regulation. In The hypothalamus, Reichlin S., Baldessarini R.J. and Martin M.B.(Eds.), Raven Press, New York,pp.303-321.

Klein E., Siegel R.A., Conforti N., Feldman S. and Chowers I.(1979): Neuroendocrine function in long-term pinealectomized male rats, following visual and audiogenic stress. J.Neural Transm. 46,113-118.

Kordon C., Héry M., Szafarczyk A., Ixart G. and Assenmacher I.(1981): Serotonin and the regulation of pituitary hormone secretion and of neuroendocrine rhythms. J.Physiol.(Paris) 77,489-496.

Menaker M., Takahashi J.S. and Eskin A.(1978): The physiology of circadian pacemakers. Ann.Rev.Physiol. 40,501-526.

Mess B., Tima L. and Trentini G.P.(1973): The role of pineal principles in ovulation. Prog. Brain Res. 39,251-259.

Mess B., Trentini G.P., Rúzsás C. and De Gaetani C.(1979): Possible role of the brain serotoninergic neuron system in inducing ovulation in the light-induced constant estrous anovulatory syndrome. In Recent advances in reproduction and regulation of fertility, Talwar G.P.(Ed.), Elsevier, North Holland Biomed.Press, pp.37-43.

Mess B., De Gaetani C., Rúzsás C. and Trentini G.P.(1981): Possible mechanisms by which the pineal gland influences reproductive function.In The pineal organ: photobiology-biochronometry-endocrinology, Oksche A.and Pévet P.(Eds.), Elsevier, North Holland Biomed.Press, pp.303-314.

Mess B., Rúzsás C. and Trentini G.P.(1984): Pineal mechanisms regulating reproduction in seasonal and non-seasonal breeders: a comparative survey. Neuroendocrinol.Lett., in press.

Meyer D.C.and Quay W.B.(1976): Effects of continuous light and darkness, and of pinealectomy, adrenalectomy and gonadectomy on uptake of 3H-serotonin by the suprachiasmatic nuclear region of male rats. Neuroendocrinology 22,231-239.
Mozskowska A., Kordon C. and Ebels I.(1974): Biochemical fractions and mechanisms involved in the pineal modulation of pituitary gonadotropin release. In The pineal gland, Wolstenholme G.E.W. and Knight J.(Eds), Churchill Livingstone, London, pp.241-258.
Motta M., Fraschini F. and Martini L.(1967): Endocrine effects of pineal gland and of melatonin. Proc.Soc.exptl.Biol.Med. 126,431-435.
Morimoto Y. and Yamamura Y.(1979): Regulation of circadian adrenocortical periodicities and of eating-fasting cycles in rats under various lighting conditions. In Biological rhythms and their central mechanism, Suda M., Hayaishi O. and Nakagawa H.(Eds.), Elsevier, North Holland, pp.177-188.
Moore R.Y. (1978): Central neural control of circadian rhythms. In Frontiers in neuroendocrinology, Ganong W.F. and Martini L.(Eds.), Vol.5, Raven Press, New York, pp185-206.
Moore R.Y. and Eichler V.B.(1972):Loss of circadian adrenal corticosterone rhythm following suprachiasmatic lesion in the rat. Brain Res. 42,201-206.
Moore R.Y. and Klein D.C.(1974): Visual pathways and the central neural control of the circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Res. 71,17-31.
Nagai K., Nishio T., Nakagawa H., Nakamura S. and Fukuda Y.(1978): Effect of bilateral lesions of the suprachiasmatic nuclei on the circadian rhythm of food-intake. Brain Res. 142,384-389.
Niles L.P., Wong Y.W., Mishra R.K. and Brown G.M. (1979): Melatonin receptors in brain. Europ.J.Pharmacol. 55,219-220.
Niles L.P.,Brown G.M.and Mishra R.K.(1983):Effects of blinding and pinealectomy on regional brain monoamine concentrations.J.Neurosci.Res.10,53-60.
Olcese J.M. and de Vlaming V.L.(1979): Daily variations of photoperiod and pinealectomy effects on hypothalamic monoamine oxidase activity in the goldfish. Comp.Biochem.Physiol. 63C,363-367.
Olcese J.M., Hall T.R., Figueroa H.R. and de Vlaming V.L.(1981): Pinealectomy and melatonin effects on daily variations of the hypothalamic serotonergic system in the goldfish. Comp.Biochem.Physiol. 70A,69-72.
Pévet P. and Haldar-Misra C.(1982): Effect of 5-methoxytryptamine on testicular atrophy induced by experimental or natural short photoperiod in the golden hamster (Mesocricetus auratus). J.Neural Transm. 55,69-84.
Pucilowski O. and Kostowski W.(1983): Aggressive behaviour and the central serotonergic systems. Behav.Brain Res. 9,33-48.
Redman J., Armstrong S. and Ng K.T.(1983): Free running activity rhythms in the rat: entrainment by melatonin. Science 219,1089-1091.
Reiter R.J.(1980): The pineal and its hormone in the control of reproduction in mammals. Endocr.Rev. 1,109-131.
Relkin R.(1978): Use of melatonin and synthetic TRH to determine site of inhibition of TSH secretion. Neuroendocrinology 25,310-313.
Richardson B.A., Vaughan M.K., Petterborg L.J., Johnson L.Y., King,T.S., Smith I. and Reiter R.J.(1983): Natural and synthetic analogues of

melatonin and related compounds.I.Effects on the reproductive system of the male syrian hamster. J.Neural Transm. 56,187-197.
Ronnekleiv O.K. and McCann S.M.(1978): Growth hormone release in conscious pinealectomized and sham operated male rats. Endocrinology 102,1694-1701.
Ruzsas C., De Gaetani C., Criscuolo M. and Trentini G.P.(1982): Effect of selective 5,7-dihydroxytryptamine lesions of the brain serotonergic neuron system on the ovulation maintaining effect of melatonin in rats exposed to continuous light. Neuroendocrinol.Lett. 4,313-322.
Sackman J.W., Little J.C., Rudeen P.K., Warring P.J. and Reiter R.J. (1977): The effects of pineal indoles given late in the light period on the reproductive organs and pituitary prolactin levels in male golden hamsters. Horm. Res. 8,84-92.
Sarda N., Coindet J., Gharib A. and Jouvet M.(1983): A comparison of the effects of S-adenosyl-l-homocysteine on sleep in normal and pineal-ectomized rats. Electroencephal.clin.Neurophysiol. 56,467-472.
Smith J.(1983): Indoles of pineal origin: biochemical and physiological status. Psychoneuroendocrinology 8,41-60.
Smythe G.A. and Lazarus L.(1974a): Suppression of human growth hormone secretion by melatonin and cyproheptadine. J.Clin.Invest. 54,116-121.
Smythe G.A. and Lazarus L.(1974b): Growth hormone responses to melatonin in man. Science 184,1373-1374.
Smythe G.A., Bradshaw J.E., Cai W.Y. and Symans R.G.(1982): Hypothalamic serotoninergic stimulation of thyrotropin secretion and related brain hormone and drug interactions in the rat. Endocrinology 111,1181-1191.
Steger R.W., Reiter R.J. and Siler-Khodr T.M.(1984): Interactions of pinealectomy and short-photoperiod exposure on the neuroendocrine axis of the male syrian hamster. Neuroendocrinology 38,158-163.
Steinbusch H.W.M.(1981): Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6,557-618.
Stephan F.K. and Zucker I.(1972): Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc.Nat.Acad.Sci.USA 69,1538-1586.
Sugden D. and Morris R.D.(1979): Changes in regional brain levels of tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, dopamine and noradrenaline after pinealectomy in the rat. J.Neurochem. 32,1593-1595.
Szafarczyk A.,Alonso G.,Ixart G.,Malaval F.,Nouguier-Soule J. and Assen-macher I.(1980): The serotoninergic system and the circadian rhythms of ACTH and corticosterone in rats. Amer.J.Physiol. 239,E482-489.
Tamarkin L., Westrom W., Hamill A. and Goldman B.(1976): Effect of melatonin on the reproductive systems of male and female syrian hamsters: a diurnal rhythm in sensitivity to melatonin. Endocrinology 99,1534-1541.
Tima L., Trentini G.P. and Mess B.(1973): Effect of serotonin on ovulation induced by pinealectomy in anovulatory frontal-deafferented rats. Neuroendocrinology 12,149-152.
Trentini G.P., Botticelli A., Barbanti Silva C. and De Gaetani C.F.(1967): Importanza dell'innervazione simpatica nel meccanismo di fee-back epifisi--ipofisario. Bioch.Biol.Sper. 6,251-258.

Trentini G.P., Tima L. and Mess B.(1973): Effect of bilateral cervical sympathectomy on ovulation and fertility of rats bearing frontal hypothalamic deafferentation and anovulatory syndrome. Horm.Res. 4,349-359.
Trentini G.P., Tima L., De Gaetani C.F. and Mess B.(1974): Luteinization induced by p-chlorophenylalanine treatment in constant oestrous anovulatory rats. Steroid Lipids Res. 5,262-267.
Trentini G.P., De Gaetani C.F., Martini L. and Mess B.(1976): Effect of pinealectomy and bilateral cervical ganglionectomy on serum LH levels in constant estrous-anovulatory rats. Proc.Soc.exptl.Biol.Med. 153,490-494.
Trentini G.P., Mess B., De Gaetani C.F. and Rúzsás C.(1978): Effect of melatonin on induction of ovulation in the light-induced constant estrous-anovulatory syndrome and possible role of the brain serotoninergic system. J.Endocrinol.Invest. 1,305-310.
Trentini G.P., Mess B., De Gaetani C.F. and Rúzsás C.(1979): Pineal-brain relationship. Prog.Brain Res. 52,341-365.
Trentini G.P., De Gaetani C.F., Criscuolo M., Balemans M.G.M., Vaessen L.M.B. and Smith I.(1982): The effect of melatonin and other indole derivatives in maintaining ovulation in rats kept in continuous light and the influence of these indoles on HIOMT activity in the pineal gland. J.Neural Transm. 53,305-314.
Trentini G.P., De Gaetani C.F., Balemans M.G.M. and Criscuolo M.(1983): The influence of melatonin and other indole derivatives on HIOMT activity of pineal gland of rats exposed to continuous light. XIV Acta Endocr. Congr.,Satellite Symp. on the role of melatonin and other 5-methoxyindoles in endocrinology and psychiatry. Stockholm, June 26, (Abstr.).
Trentini G.P., De Gaetani C.F., Rúzsás C. and Criscuolo M.(In press): Environmental light, pineal gland and regulation of ovulation. 9th Int. Conf. on Comp.Endocrinol., Hong Kong Univ.Press.
Urry R.L. and Ellis L.C.(1975): Monoamine oxidase (MAO) activity of the hypothalamus and pituitary: alterations after pinealectomy, changes in photoperiod, or additions of melatonin in vitro. Experientia 15,891-892.
Vacas M.I. and Cardinali D.P.(1979): Diurnal changes in melatonin binding sites of hamster and rat brains. Correlation with neuroendocrine responsiveness to melatonin. Neurosci.Lett.15,259-263.
Valzelli L., Bernasconi S. and Cusumano G.(1977):Annual and daily changes in brain serotonin content in differentially housed mice. Neuropsychobiology 3,35-41.
Vaughan M.K.(1981): The pineal gland: a survey of its antigonadotrophic substances and their actions. Int.Rev.Physiol.24,41-85.
Vaughan M.K., Vaughan G.M., Reiter R.J.and Benson B.(1972): Effect of melatonin and other pineal indoles on adrenal enlargement produced in male and female mice by pinealectomy, unilateral adrenalectomy, castration, and cold stress. Neuroendocrinology 10,139-154.
Vaughan M.K.,Powanda M.C.,Brainard G.C.,Johnson L.Y. and Reiter R.J.(1982): Effects of blinding or afternoon melatonin injection on plasma cholesterol, triglycerides, glucose, TSH and thyroid hormone levels in male and female syrian hamsters. In The pineal and its hormones,Reiter R.J.(Ed.),Alan Liss,New York,pp.177-186.

Vaughan M.K., Richardson B.A., Johnson L.Y., Petterborg L.J., Powanda M.C. Reiter R.J. and Smith I.(1983): Natural and synthetic analogues of melatonin and related compounds. II. Effects on plasma thyroid hormones and cholesterol levels in male syrian hamsters. J.Neural Transm. 56,279-291.

Vriend J.and Gibbs F.P.(1984): Coincidence of counter-antigonadal and counter-antithyroid action of melatonin administration via the drinking water in male golden hamsters. Life Sci. 34,617-623.

Walker R.F.(1982a): Reinstatement of LH surges by serotonin neuroleptics in aging, constant estrous rats. Neurobiol.Aging 3,253-257.

Walker R.F.(1982b): Melatonin:serotonin interaction during termination of the LH surge in rats. In The pineal and its hormones, Reiter R.J.(Ed.), Alan Liss, New York,pp.167-176.

Walker R.F.(1983): Quantitative and temporal aspects of serotonin's facilitatory action on phasic secretion of luteinizing hormone in female rats. Neuroendocrinology 36,468-474.

Watts A.G. and Fink G.(1981): Effects of short-term constant light on the proestrous luteinizing hormone surge and pituitary responsiveness in the female rat. Neuroendocrinology 33,176-180.

Williams J.H., Miall-Allen V.M., Klinowski M. and Azmitia E.C.(1983): Effects of microinjection of 5,7-dihydroxytryptamine in the suprachiasmatic nuclei of the rat in serotonin reuptake and the circadian variaton of corticosterone levels. Neuroendocrinology 36,431-435.

Willoughby J.O, Audet J., Crossfield T. and Martin J.B.(1978): Growth hormone and prolactin: effects of pinealectomy. Proc.Aust.Physiol. Pharmacol.Soc. 9,1988.

Wurtman R.J., Altschule M.D. and Holmgren U.(1959): Effect of pinealectomy and of bovine pineal extract in rats. Amer.J.Physiol. 197,108-110.

Wurtman R.J., Roth W., Altschule M.D. and Wurtman J.J.(1961): Interactions of the pineal and exposure to continuous light on organ weights of female rats. Acta Endocr.36,617-624.

Wurtman R.J., Axelrod J.and Chu E.W.(1963): Melatonin, a pineal substance effect on the rat ovary. Science 141,277-278.

Yates C.A. and Herbert J.(1976): Differential circadian rhythms in pineal and hypothalamic 5-HT induced by artificial photoperiods or melatonin. Nature (Lond.) 262,219-220.

Yates C.A. and Herbert J.(1979): The effects of different photoperiod on circadian 5-HT rhythms in regional brain areas and their modulation by pinealectomy, melatonin and oestradiol. Brain Res. 176,311-326.

Zanoboni A. and Zanoboni-Muciaccia W.(1967): Experimental hypertension in pinealectomized rats. Life Sci 6,2327-2330.

The Pineal Gland
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INTERACTIONS BETWEEN NON-SEROTONERGIC NEUROTRANSMITTERS AND MELATONIN IN THE CONTROL OF REPRODUCTIVE ACTIVITY IN THE RAT

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INTRODUCTION

The pineal gland mediates the inhibitory, as well as the stimulatory influences of the photoperiod on gonadal activity in mammalian species /for review: Hoffmann 1979, 1981; Reiter 1980/. During the last few years, the efforts of our working group have been devoted to the identification of the neural pathways through which the pineal gland transmits the photoperiodic information to the hypothalamo - pituitary - gonadal system in the rat. Our data have indicated that the serotonergic system represents an important link in the mechanism by which the pineal hormones /at least melatonin/ influence gonadal activity.

The present work attempts to review the new experimental evidence suggesting a possible relationship between the pineal gland and other monoaminergic, non-serotonergic neurotransmitter mechanisms in the regulation of the gonadal system.

Since the pineal gland seems to represent a modulator of the neuroendocrine circuits rather than a basic control mechanism /Mess et al. 1981, 1983/, it may be assumed that the pineal hormones influence reproductive function in the rat via brain neurotransmitter systems.

In our experimental model, the constant estrous-anovulatory rat, the Luteinizing Hormone /LH/-release mechanism is damaged due to a deficiency of these presumed neurotransmitter stimuli.

IMPORTANCE OF THE SUPRACHIASMATIC NUCLEI / SCN / IN THE ORGANIZATION OF CYCLIC NEUROENDOCRINE PROCESSES

The timing of the phasic release of LH, with respect to the photoperiod, requires a complex organization that guarantees the appropriate response to changes in environmental conditions.

It is necessary to underscore the crucial role of the SCN in the organization of cyclic neuroendocrine rhythms that presumably are coupled with the day-night cycle. Several studies suggest that the SCN are responsible for the synchronization of gonadal activity with environmental lighting conditions /Meyer and Quay 1976; Turek et al. 1983/ without involving the pineal gland. On the other hand, the SCN

also are involved in the generation of daily rhythms within the pineal gland, since the ablation of this brain region abolishes the circadian rhythmicity of melatonin production /Moore 1974; Moore and Klein 1974/. Light impulses pass to the SCN through the retinohypothalamic pathway /Moore et al. 1967; Moore and Lenn 1972; Moore and Eichler 1976/. Since pineal secretion is regulated by the SCN via a projection of sympathetic fibres /Kappers 1965; Moore 1978/, the pineal hormones provide the neural signal that transmits the photic response to the hypothalamo - gonadal system /Bittman et al. 1979, 1983; Rusak 1980/. Changes in the photoperiod, acting via the SCN, alter the rhythm of melatonin secretion. The SCN have been demonstrated to contribute to the suppression of melatonin synthesis due to continuous light exposure /Nishino et al. 1976/.

Furthermore, the pineal gland and SCN seem to be reciprocally connected. The high affinity binding of melatonin by the SCN suggests the presence of specific receptors for melatonin in this region /Cardinali et al. 1979; Niles et al. 1979/. Immunohistochemical studies /Bubenik et al. 1976/, implants or microinjections of melatonin into the SCN /Glass and Lynch 1981, 1982/ also confirmed the hypothesis that the SCN are an important target area for melatonin.

Fig. 1. represents the presumed reciprocal connections between the SCN and the pineal gland in the regulation of pituitary hormone secretion.

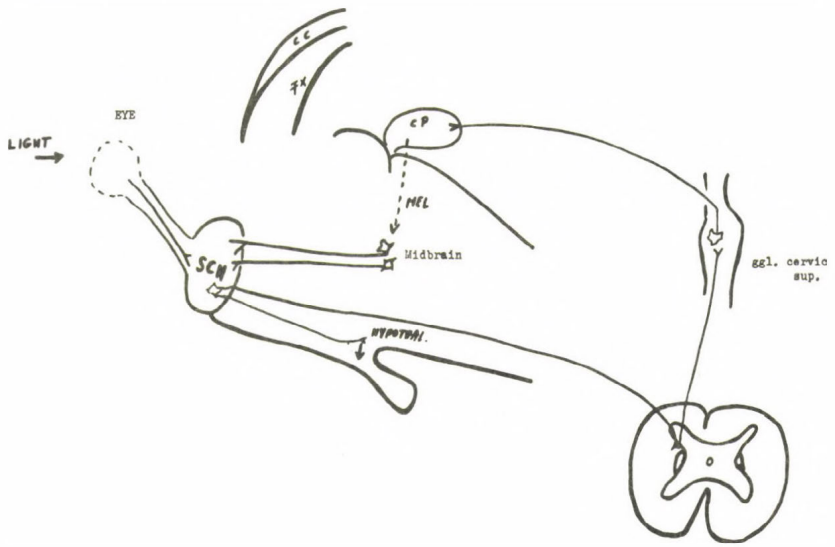

Fig. 1. Possible routes and connections between pineal gland and SCN in mediating light impulses towards pituitary hormone secretion.

Abbreviations: CC: corpus callosum
Fx: fornix

The a forementioned data stimulated us to investigate the significance of the fact that the SCN receive a large aboundance of nerve terminals originating from different neurotransmitter systems /Fuxe 1965; Saavedra et al. 1974a,b; Van de Kar and Lorens 1979/. These latter morphological findings seem to be in accordance with our previously proposed hypothesis that the pineal hormones exert their action on monoaminergic pathways /Mess et al. 1981, 1983/ terminating in the SCN that control pituitary LH release /Rúzsás et al. 1983/.

Previously, the attention of our group was focused upon the importance of the serotonergic connections of the SCN in mediating the action of melatonin in terms ovarian cyclicity in the rat /Trentini et al. 1979; Rúzsás et al. 1981, 1982, 1983/.

However, the SCN also were demonstrated to contain high concentrations of catecholamines, both norepinephrine / NE / and dopamine / DA /, according to the data of Palkovits et al. /1974/. Daily rhythms in the number of α- and β-adrenergic and dopaminergic receptors regulated by the SCN also were documented. The ablation of the SCN abolishes these circadian rhythms /Kafka et al. 1983/.

These data suggest that the SCN might act via different neurotransmitter systems, other than serotonin, organizing the rhythmicity of neuroendocrine events that are associated with the light-dark cycle.

DATA SUGGESTING THE POSSIBLE INVOLVEMENT OF CATECHOLAMINES IN THE MELATONIN CONTROL OF OVULATION

In order to better understand the complex pineal mechanisms regulating reproductive processes in the rat, in our further experiments, the possible role of non-serotonergic brain neurotransmitters was investigated.

As was mentioned in the introduction, serotonin has been proved to be an important but not necessarily the single regulator of cyclic gonadotrophin release in the rat. Clear temporal relationship was demonstrated between increased NE turnover rates in the median eminence / ME / and the preovulatory discharge of LH into the plasma /Homma and Wuttke 1980/. Thus, NE seems to initiate the discharge of gonadotrophins by evoking the release of LHRH from the nerve terminals of the ME into the portal circulation. A dramatic increase in NE turnover in the SCN was detected at the time of the preovulatory LH surge /Rance and Barraclough 1981; Rance et al. 1981/ that could be prevented by the α-adrenergic blocker, clonidine /Coen and Coombs 1983/.

The role of DA in the regulation of gonadotrophins is less clear. It seems to be inhibitory under certain conditions /Sawyer et al.1974/, however, other studies failed to demonstrate any conclusive effect of DA on LH secretion /Rubinstein and Sawyer 1970; Tima and Flerkó 1974; Kimura et al. 1975/. The data of Negro-Vilar /1979/, however, appear to indicate a permissive role of DA in the ovulatory cycle, similar to that of serotonin, as described previously /Rúzsás et al. 1983/.

The involvement of catecholamines in the regulation of the LH surge becomes even more evident in certain types of the anovulatory syndrome in the rat which are considerations in our investigations.

Decreased hypothalamic catecholamine turnover rates were detected in the rat rendered anovulatory by neonatal androgenization /Lookingland et al. 1982/. According to the data of Tima and Flerkó /1974/, the lack of ovulation in the constant estrous-anovulatory rat appears

to be the consequence of the deficiency of NE stimuli for the release of LHRH.

In contrast, Fuxe et al./1972/ reported the persistence of high DA turnover in the hypothalamus of both the androgen sterilized rat and the rat exposed to continuous illumination / LL /.

Information generated during the last few years suggests the possibility that the pineal hormones are able to modulate the turnover of brain neurotransmitters other than serotonin. A reduction in the activity of DA tuberoinfundibular neurons was demonstrated in the blind-anosmic-, as well as in the pinealectomized rat /Morgan and Reiter 1982/. Recently, the group of Reiter /1984/ reported changes in hypothalamic NE and DA turnover as a consequence of photoperiod manipulations that influence pineal activity. According to these experiments, the short photoperiod-induced gonadal atrophy was associated with significant reduction of NE and DA turnover in the ME of the Syrian hamster. Pinealectomy reversed the decrease in NE turnover which was supposed to be responsible for the lack of cyclic gonadotrophin release. In the Syrian hamster, hypothalamic serotonin content was not affected by the short photoperiod. In contrast, under LL exposure /extremely long, constant photoperiod/, Trentini et al. /1981/ demonstrated the decrease in hypothalamic serotonin turnover in the rat that was reversed by daily melatonin administration.

The recent data of Sallanon et al. /1982/ also suggest the existence of new target areas for melatonin in the brain. Using double RIA techniques, these authors could detect radioimmunoreactive melatonin in the A_1, A_2 groups of catecholaminergic cells in the medulla oblongata, in accordance with the earlier results of Kopp et al. /1980/. Surprisingly, however, these authors failed to demonstrate immunoreactive melatonin in the midbrain raphe nuclei. This latter finding is in contrast with earlier results /Fraschini et al. 1968; Cardinali et al. 1979; Niles et al. 1979/ considering that this region is the main area of specific melatonin receptors.

There is considerable evidence suggesting a possible modulatory influence of melatonin on DA neurotransmission which might be also responsible for the activity of melatonin to influence the neuroendocrine system. Subnanomolar concentrations /10^{-7}, 10^{-8} M/ of melatonin were demonstrated to inhibit DA release from hypothalamic tissue /Zisapel and Laudon, 1984/.

In the last few years, DA neurons were hypothesized to represent a photosensitive system which might be involved in the transmission of photoperiodic information to the secretory rhythm of the pineal gland /Pang et al 1980; Frederick et al. 1982; Morgan and Kamp 1983/.

CATECHOLAMINERGIC PATHWAYS OF THE BRAIN STEM IMPLICATED IN THE MEDIATION OF THE PROGONADOTROPHIC ACTION OF MELATONIN

According to our previous studies serotonergic fibers projecting from the midbrain raphe nuclei to the SCN play an important role in the mechanism by which prolonged melatonin administration restores reproductive activity in the LL exposed rat /Rúzsás et al. 1981, 1982, 1983/. Since both of these regions equally receive numerous projections from the catecholaminergic system, it seems presumable that catecholaminergic pathways might influence the activity of serotonergic neurons

thereby modifying the responsiveness of the serotonergic system to melatonin.

The possibility of an interaction between serotonergic and catecholaminergic systems seems to be supported by neuroanatomical and pharmacological evidence.

A./ Catecholaminergic connections of the midbrain raphe nuclei

Histochemical studies have detected the presence of catecholaminergic fibres in the close proximity of the serotonin-producing perikarya of the dorsal / DR / and median / MR / raphe /Roizen and Jacobowitz 1976/. These catecholaminergic terminals have been shown to enter in synaptic contact with serotonergic cell bodies. The presence of catecholamines and catecholamine-producing enzymes was also detected in the midbrain raphe nuclei. According to the studies of Saavedra et al. /1976/, DR contains a high level of NE, one-third the concentration of that of serotonin, and a small amount of DA. The influence of DA on serotonergic neurons is suggested by the fact that the DA agonist, apomorphine was demonstrated to increase serotonin content in the DR, while the DA antagonist, haloperidol provoked a remarkable decrease in the serotonin level of this nucleus /Lee and Geyer 1982/.

Serotonergic cell bodies located in the DR / B_7 cell group / and MR / B_8 cell group / receive numerous projections originating from the locus coeruleus / A_6 cell group /. This projection represents the major input to the mesencephalic raphe nuclei /Fuxe 1965; Louizou 1969; Ungerstedt 1971; Chou and Bloom 1974/. / Fig. 2. /

B./ Catecholaminergic connections of the SCN

The SCN contain high concentrations of NE and DA, in addition to serotonin / Palkovits et al. 1974 /. According to the data of Sakai et al. /1977/, obtained by the horseradish peroxidase technique, a neural pathway was identified originating from NE neurons located in the locus coeruleus projecting via the median forebrain bundle and terminating in the SCN.

SCN also receive DA fibres, although in smaller proportion, since the destruction of the ascending nigrostriatal DA system leads to a decrease in DA content of the SCN /Kizer et al. 1976/. Electrophysiological data of Nishino and Koizumi /1977/ provided evidence for the presence of both serotonin and NE sensitive neurons in the SCN. These neurotransmitters are involved in the regulation of circadian rhythms.

C./ Interaction of catecholamines and melatonin in the constant estrous-anovulatory / CEA / rat

In the present experiments, the influence of destruction of NE and DA input of the DR, MR or SCN, the presumed sites of action of melatonin, was investigated on the progonadotrophic effect of melatonin in the rat exposed to LL.

Adult female rats were submitted to discrete chemical lesions of the NE and DA connections of the DR, MR and of the SCN. 6-hydroxydopamine, the neurotoxin for catecholamine-containing neurons, was applied by local stereotaxic microinjection into the above mentioned

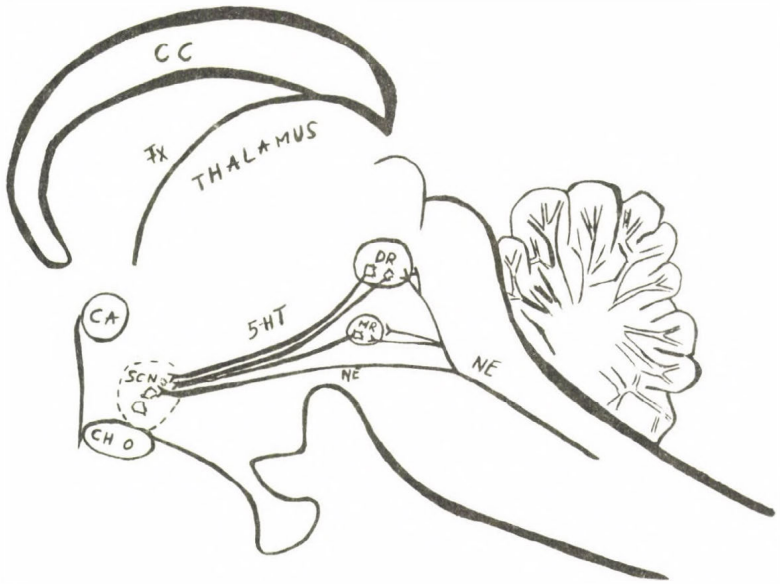

Fig. 2. Monoaminergic pathways of the brain stem.
/On the base of Ungerstedt 1971/
Abbreviations: CC: corpus callosum 5-HT: serotonin
Fx: fornix
CHO: chiasma opticum

nuclei. The neurotoxin was applied in combination with either des - methylimipramine / DMI / or benztropin, to discretely damage the NE or DA systems. DMI / 25 mg/kg, intraperitoneally / was injected 45 min prior to the application of the neurotoxin, in order to prevent the destruction of NE nerve endings, thereby enhancing the specificity of 6-hydroxy-dopamine in lesioning DA neurons. Pretreatment with benz - tropin leads to the selective injury of the NE system /Herve et al. 1982/, thereby blocking the uptake of the neurotoxin into DA terminals; the DA system thus remains protected.

Thereafter, from the first postoperative day, the rats bearing the neurotoxic lesion were exposed to LL for 4 months and treated daily with 10 ug melatonin. Vaginal smears and occurrence of corpora lutea were recorded.

α ./ Neurotoxic lesion of the NE system

Neurotoxic injury of the NE terminals within the investigated brain areas led to the results summarized in Fig. 3. and Table 1.

Fig. 3. demonstrates the effect of NE terminal injury on the % incidence of rats showing vaginal cyclicity. Under normal light - dark conditions / 14:10 /, the cyclicity remained preserved in nearly

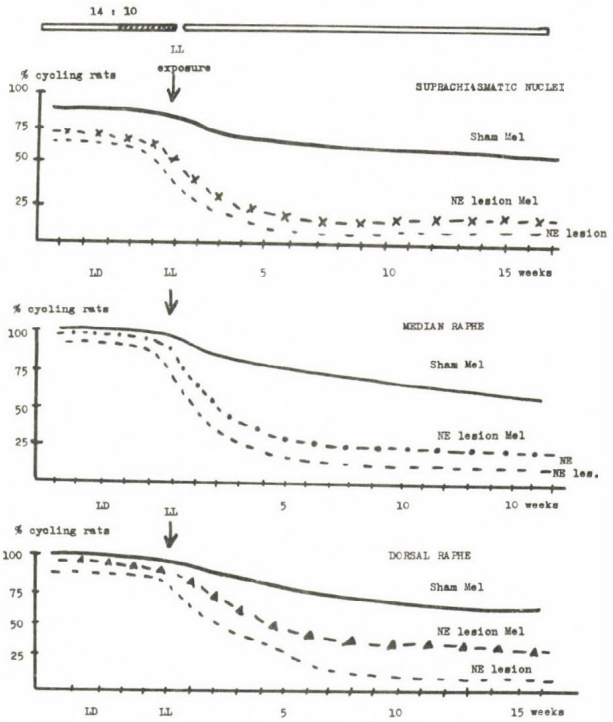

Fig. 3. Percentage occurrence of normal cyclicity in continuous light / LL / exposed rats bearing neurotoxic lesion of norepinephrinergic / NE / terminals in different brain areas with or without melatonin / Mel / treatment.

90% of animals lesioned in the DR and MR. SCN lesions reduced the occurrence of cycling rats to 75%. None of the applied lesions al - tered the effect of LL exposure upon the vaginal cycle as indicated by the significantly lowered proportion of cycling rats in each group /dotted lines/. In the sham operated LL exposed groups /heavy lines/, daily melatonin treatment maintained the cyclicity in about 70% of animals. Degeneration of NE connections of DR slightly reduced the effect of melatonin. In contrast, lesion of MR markedly counteracted the effect of melatonin; this effect was even more pronounced when NE nerve endings of SCN were destroyed.

Table 1. demonstrates the presence of corpora lutea in the ovaries of animals bearing lesions of the NE terminals in the differ- ent brain regions. Under LD conditions, injury of the SCN slightly re- duced the ovulatory frequency, while midbrain raphe lesions were not influential in this respect. LL exposure prevented luteinization in each type of the lesion bearing groups, similarly to the sham oper - ated animals. In the sham operated group, melatonin treatment main- tained luteinization in more than 70% of animals / $p < 0{,}01$ /, in con-

trast to LL exposure alone. Destruction of NE connections of MR and SCN prevented the luteinization inducing effect of melatonin, as evidenced by the significant reduction in the % of rats showing luteinization, compared to the sham operated melatonin treated group. Lesion of NE terminals localized in DR failed to influence the effect of melatonin.

Table 1.

EFFECT OF NEUROTOXIC LESION OF THE NOREPINEPHRINERGIC NERVE TERMINALS LOCATED IN DIFFERENT BRAIN AREAS ON THE OCCURRENCE OF CORPORA LUTEA IN RATS EXPOSED TO CONTINUOUS LIGHT / LL / AND TREATED WITH MELATONIN

Experimental group	Occurrence of corpora lutea No / Total	%	Statistical significance
L D			
1. Sham	9 / 10	90,0	
2. Suprachiasmatic nuclei	5 / 8	62,5	
3. Median raphe	8 / 10	80,0	
4. Dorsal raphe	8 / 10	80,0	
L L			
5. Sham	1 / 12	8,3	$p < 0,01$ vs. gr. 1
6. Suprachiasmatic nuclei	0 / 8	0,0	$p < 0,05$ vs. gr. 2
7. Median raphe	0 / 10	0,0	$p < 0,01$ vs. gr. 3
8. Dorsal raphe	0 / 7	0,0	$p < 0,01$ vs gr. 4
L L + Melatonin			
9. Sham	10 / 14	71,4	$p < 0,01$ vs. gr. 5
10. Suprachiasmatic nuclei	1 / 12	8,3	$p < 0,01$ vs. gr. 9 and vs. gr. 2
11. Median raphe	1 / 9	11,1	$p < 0,01$ vs. gr. 9
12. Dorsal raphe	5 / 8	62,5	and vs. gr. 3

β ./ Neurotoxic lesion of the DA system

Lesions of the DA terminals in the same brain areas gave mainly negative results. Pharmacological injury of the DA system, in any type of localization, failed to inhibit the effect of melatonin exerted upon the gonadal system.

Data of these series are summarized in Table 2.

These results show that the DA system is not influential on the effect of melatonin exerted upon the formation of corpora lutea.

Table 2.

EFFECT OF DAILY MELATONIN TREATMENT ON THE FORMATION OF CORPORA LUTEA AND ON THE INCIDENCE OF THE ESTROUS PHASE IN THE VAGINAL CYCLE IN LL EXPOSED RATS BEARING NEUROTOXIC LESION OF DOPAMINERGIC CONNECTIONS OF DIFFERENT BRAIN AREAS

Experimental group	Occurrence of luteinization No / Total	%	% incidence of estrous phase
LL + Melatonin			
Sham	8 / 12	66,7	32,7
DR	6 / 12	50,0	74,2^{x}
MR	4 / 10	40,0	69,2^{x}
SCN	4 / 12	33,3	65,9^{x}

x $p < 0,05$ vs.sham

However, lesions of DA terminals resulted in a considerable prolongation of the estrous phase of the vaginal cycle, compared to melatonin treated, sham operated rats, independently of the localization of the neurotoxic lesion. These latter data suggest that a minimal level of DA might be required for the effectiveness of exogenous melatonin.

Fig. 4. shows the effect of specific damage of NE and DA connections of the investigated brain areas compared to the effect of selective chemical lesion of the serotonergic system /by 5,7-dihydroxy - tryptamine/ in the same localization. Destruction of NE and serotonergic terminals of the SCN proved to be equally effective in preventing the luteinizing action of melatonin. These results suggest the possibility that NE and serotonergic terminals of the SCN may be equally involved in the regulation of cyclic gonadotrophin release activated by melatonin under LL exposure. Chemical injury of NE nerve endings of MR significantly prevented the effect of melatonin, com - pared not only to the sham operated, but also to the 5,7-dihydroxy-tryptamine / 5,7-DHT / lesion bearing group. These data lead to the assumption that NE connections of MR could modify the effect of melatonin's influence on the serotonergic pathways originating from this brain region. Melatonin's effect was not influenced by any type of neurotoxic lesion localized in the DR. Neurotoxic lesion of the DA terminals was significantly less effective than similar destruction of the NE or serotonergic system.

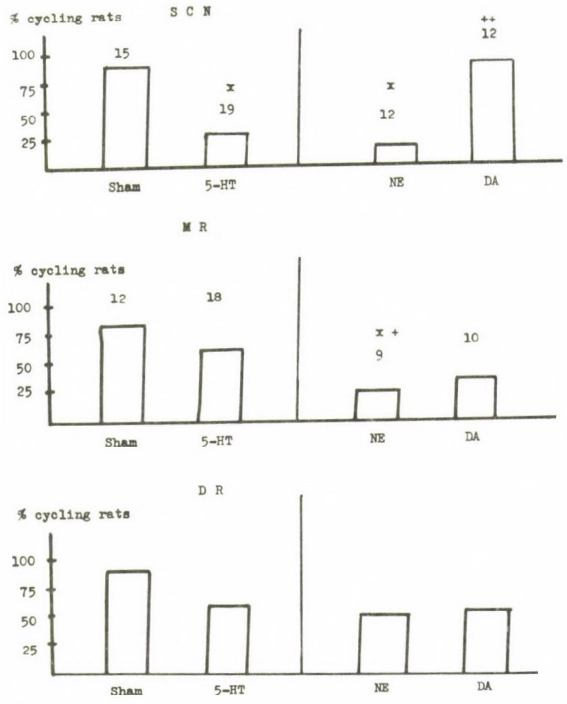

Fig. 4. Effect of melatonin treatment on the incidence of luteinization in LL - exposed rats bearing separate lesions of the serotonergic,/ 5-HT /, norepinephrinergic / NE / and dopaminergic / DA / terminals in SCN, median and dorsal raphe nuclei.

x $p < 0,05$ vs. sham
x $p < 0,05$ vs. corresponding 5-HT lesioned group
x $p < 0,05$ vs. corresponding 5-HT lesioned and NE lesioned group

As the final step of these investigations, the ovulation-inducing potency of NE, introduced into the brain ventricular system, was recorded in melatonin-treated LL-exposed rats bearing lesions of the NE terminals in the investigated brain areas. This experimental series was undertaken in order to investigate whether the ovulation - inducing effect of melatonin could be restored by substitution of the neurotoxin-induced reduction of NE activity.

Fig. 5. demonstrates the changes in the ovulatory response to increasing concentrations of NE in the brain of melatonin-treated groups. In these series, the identification of the presence of tubal ova /after dissecting the oviduct in the estrous phase/ was considered as the index of ovulation.

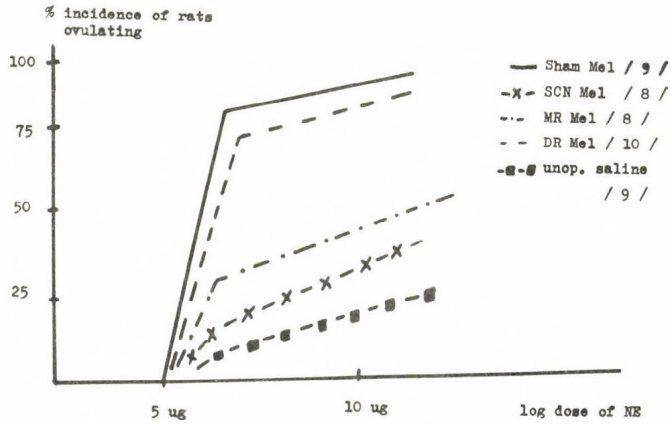

Fig. 5. Induction of ovulation by increasing doses of NE, injected intraventricularly in melatonin - treated LL - exposed rats bearing chemical lesions of norepinephrinergic nerve endings located in SCN, median or dorsal / MR; DR / raphe nuclei.

In the sham-operated melatonin-treated groups, enhanced sensitivity to NE was observed compared to the depressed reactivity of the saline-treated LL-exposed rats. In this latter group, 5 µg of NE was weakly effective in affecting the ovulatory response only the higher dose of NE resulted in a low percentage of ovulation. In DR lesioned, melatonin-treated rats, the sensitivity to NE was preserved /only slightly diminished compared to sham-operated group/, while the reactivity to NE was significantly lower in the MR lesioned group /$p < 0,01$/. The responsiveness to NE was even more diminished in SCN lesioned rats: these animals are nearly unable to respond to the low dose: only the higher dose of NE provoked ovulation with very low frequency.

Since the SCN seem to play a prominent role in the mechanism responsible for the release of LHRH subserving ovulation, the destruction of this final link apparently renders ineffective all the modulatory influences, including the pineal mechanisms. On the other hand, NE connections of other brain regions, like MR, - on the basis of our data - might specifically interact with influences derived from the pineal gland.

CONCLUSIONS AND SUMMARY OF THE RESULTS

The present work was undertaken in an attempt to survey the monoaminergic neurotransmitter mechanisms by which the pineal gland controls reproductive function in the rat.

1. The present results confirm the primarily role of the serotonergic system in the mediation of the progonadotrophic action of melatonin as proposed previously /Trentini et al. 1981; Rúzsás et al. 1983/.

2. The data seem to negate the involvement of the DA system in mediating the progonadotrophic action of melatonin under the investigated conditions.

3. These experiments appear to indicate that the NE system, similarly to the serotonergic neurons, is effective in transmitting the effect of pineal hormones on reproductive activity. On the basis of these results, a NE mechanism, acting through the SCN, may also operate as the final link in the elicitation of the LH surge, activated by melatonin.

Our previous proposal that serotonin represents the main mediator of pineal activity controlling the reproductive system seems to be in accordance with data obtained in vitro /Cardinali et al. 1975/. In these studies, melatonin was 2-3 times more effective in inhibiting hypothalamic serotonin accumulation than that of catecholamines. These findings confirm that melatonin may affect neurotransmitter uptake rather in serotonin, than in catecholamine-containing neurons.

4. The present results raise the possibility that, besides serotonergic projections, the NE afferents of the SCN contribute to the activation of this important control center organizing circadian rhythms and consequently, the cyclic rhythm of ovulation.

Serotonergic connections of the SCN were previously shown to be a critical component of the neural pathways mediating the progonadotrophic action of melatonin in the LL-exposed rat /Rúzsás et al. 1981, 1982, 1983/. Melatonin, on the other hand, may interfere, under certain circumstances, with other non-serotonergic neurotransmitters in influencing the rhythmic release of LH. This NE mechanism probably may control the activity of serotonergic pathways that might be important in the organization of LH release. Based on these results, theoretically, it can be assumed that a critical balance between the serotonergic and NE systems is required for the ovulatory discharge of LH affected by melatonin.

These data raise the possibility, therefore, that NE could act as co-transmitter with serotonin in eliciting the release of LHRH stimulated by melatonin. This is, at present, only a working hypothesis that requires further experimental confirmation.

5. The review of the data seems to promote the following concept:

Melatonin modulates the circadian organization of neuroendocrine rhythms in the rat, influencing the activity of the SCN that receive inputs from different components of the monoaminergic systems terminating in these nuclei.

This complex system appears to be involved in the mediation of environmental light impulses subserving reproductive activity. Light informations received from the eyes presumably are integrated by the SCN. The hypothalamo - pituitary system utilizes light information for the timing of the LH surge through neurotransmitter signals originating from the SCN. The information about daylength also is transferred to the pineal gland with the involvement of the SCN, via activation of the sympathetic nervous system. The modification of

pineal hormone secretion influences the activity of the serotonergic system /Anton-Tay et al. 1968; Trentini et al. 1979; 1981/ as indicated by previous data.

However, based on the present results and those from the literature, NE projections of the serotonergic raphe system and of the SCN might also be involved in the mechanism by which melatonin influences the activity of the SCN.

Fig. 5. gives a schematic summary of this postulated mechanism.

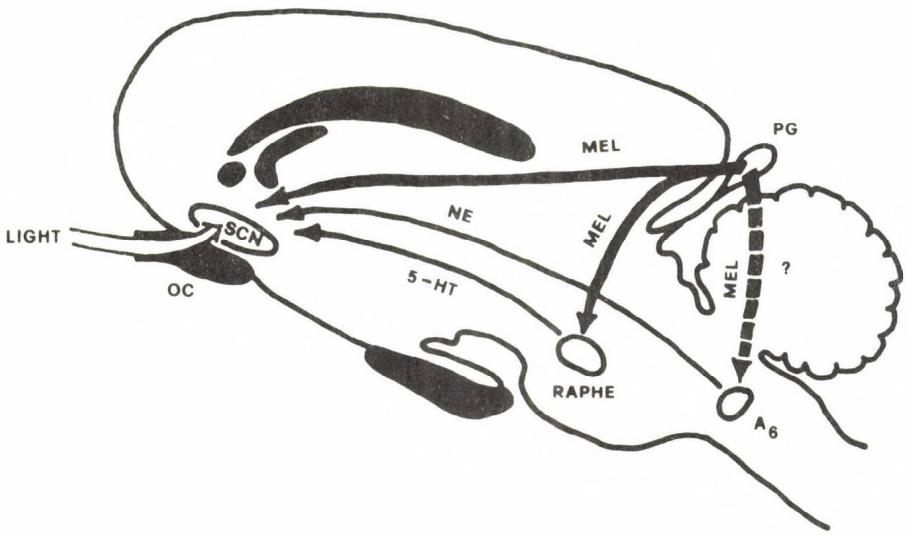

Fig. 5. Postulated role of brain neurotransmitters involved in the regulatory mechanism by which melatonin influences the activity of the suprachiasmatic nuclei.

Abbreviations:

PG: pineal gland
OC: optic chiasma
A_6: norepinephrinergic cell group
NE: Noradrenergic tract
5-HT: Serotonergic tract
MEL: melatonin
SCN: suprachiasmatic nuclei

Accordingly, it can be inferred that melatonin might interact with NE and serotonergic receptors located in the SCN giving rise to the neural signal essential for the release of LHRH. However, it seems probable that this complex system may involve even more components in the organization of rhythmic neuroendocrine events. Our data raise the possibility that pineal hormones contribute to the phasic release of LH not exclusively via serotonergic pathways. NE seems to be required for the manifestation of the progonadotrophic effect of

melatonin. The modification of NE content of the SCN might be probably the final step in this regulatory mechanism.

Speculatively, the hypothesis that melatonin may interact with different neurotransmitters, could provide a possible explanation of melatonin's multiple effects on reproduction.

The heuristic synthesis of these conflicting and controversial data underscores the concept that the mechanism by which melatonin affects reproduction is considerably more complex than previously appreciated.

REFERENCES

Anton-Tay F., Chou C., Anton S., Wurtman R.J. /1968/: Brain serotonin concentration: elevation following intraperitoneal administration of melatonin. Science 162: 277-278

Bittman E.L., Goldman B.D., Zucker J. /1979/: Testicular responses to melatonin are altered by lesions of the suprachiasmatic nuclei in golden hamsters. Biol. Reproduction 21: 647-657

Bittman E.L., Dempsey R.J., Karsch F.J. /1983/: Pineal melatonin secretion drives the photoperiodic response to daylength in the ewe. Endocrinology 113: 2267-2283

Bubenik G.A., Brown G.M., Grota L.J. /1976/: Differential localization of N-acetylated indolealkylamines in CNS and Harderian gland using immunohistochemistry. Brain Res. 118: 417-427

Cardinali D.P., Nagle C.A., Freire F., Rosner J.N. /1975/: Effects of melatonin on neurotransmitter uptake and release by synaptosome-rich homogenates of the rat hypothalamus. Neuroendocrinology 18: 72-85

Cardinali D.P., Vacas M.I., Boyer E.E. /1979/: Specific binding of melatonin in bovine brain. Endocrinology 105: 437-441

Chu N.S., Bloom F.E. /1974/: The catecholamine-containing neurons in the cat dorsolateral pontine tegmentum: distribution of cell bodies and some axonal projection. Brain Res. 66: 1-21

Coen C.W., Coombs M.C. /1983/: Effects of manipulating catecholamines on the incidence of the preovulatory surge of Luteinizing Hormone and ovulation in the rat: evidence for a necessary involvement of hypothalamic adrenaline in the normal or "midnight" surge. Neuroendocrinology 10: 187-206

Fraschini F., Mess B., Piva F., Martini L. /1968/: Brain receptors sensitive to indole compounds: function in control of Luteinizing Hormone secretion. Science 159: 1104-1105

Frederick J.M., Rayborn M.E., Laties A.M., Lam D.M.K., Hollyfield J.G. /1982/: Dopaminergic neurons in the human retina. J. comp. Neurol. 210: 65-79

Fuxe K. /1965/: Evidence for the existence of monoamine neurons in the central nervous system IV. Distribution of monoamine-containing neurons. Acta physiol. scand. 62. Suppl. 233: 1-55

Fuxe K., Hökfelt T., Nilsson O. /1972/: Effect of constant light and androgen-sterilization on amine turnover of the tubero-infundibular dopamine neurons: Blockade of cyclic activity and induction of a persistent high DA turnover in the median eminence. Acta Endocrinol. 69: 625-639

Glass J.D., Lynch G.R. /1981/: Melatonin identification of sites of antigonadal action in mouse brain. Science 214: 821-823

Glass J.E., Lynch G.R. /1982/: Evidence for a brain site of melatonin action in the white-footed mouse, Peromyscus leucopus. Neuroendo - crinology 34: 1-6

Hoffmann K. /1979/: Photoperiod, pineal, melatonin and reproduction in hamsters. In: The pineal gland of vertebrates including man. Progr. Brain Res. 52, Ariens-Kappers J. and Pévet P. /eds./ Elsevier, Amsterdam, pp. 397-415

Hoffmann K. /1981/: The role of the pineal gland in the photoperiodic control of seasonal cycles in hamsters. In: Biological clocks in seasonal reproductive cycles. Follett B.K. and Follett D.E. /eds./ Wright, Bristol, pp. 237-250

Herve D., Blanc G., Glowinski J., Tassin J.P. /1982/: Reduction of dopamine utilization in the prefrontal cortex but not in the nucleus accumbens after selective destruction of noradrenergic fibres innervating the ventral tegmental area. Brain. Res. 237: 510-516

Homma K., Wuttke W. /1980/: Norepinephrine and dopamine turnover rates in the medial preoptic areas and the mediobasal hypothalamus of the rat brain after various endocrinological manipulations. Endocrinology 106: 1849-1854

Kafka M.S., Wirz-Justice A., Naber D., Moore R.Y., Benedito M.A. /1983/: Circadian rhythms in rat brain neurotransmitter receptors. Fed. Proc. 42: 2796-2801

Kappers A.J. /1965/: Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Progr. Brain Res. 10: 87-153

Kimura F., Sakuma Y., Kawakami M. /1976/: Role of catecholamines in the neural control of gonadotrophin secretion. Jap. J. Clin. Med. 33: 514-517

Kizer J.S., Palkovits M., Brownstein M.J. /1976/: The projection of A_8, A_9, A_{10} dopaminergic cell bodies: evidence for a nigrostriato-hypothalamic-median eminence dopaminergic pathway. Brain. Res. 362: 363-370

Kopp N., Claustrat B., Tappaz M. /1980/: Evidence for the presence of melatonin in the human brain. Neurosci. Lett. 19: 237-242

Lee E.H.Y., Geyer M.A. /1982/: Selective effects of apomorphine on dorsal raphe neurons: a cytofluorimetric study. Brain Res.Bulletin 9: 719-725

Lookingland K.J., Wise P.M., Barraclough C.A. /1982/: Failure of the hypothalamic noradrenergic system to function in adult androgen sterilized rat. Biol. Reproduction 27: 268-281

Louizou L.A. /1969/: Projections of the locus coeruleus in the albino rat. Brain Res. 15: 563-566

Mess B., Trentini G.P., Rúzsás C., De Gaetani C. /1979/: Possible role of the brain serotonergic neuron system in inducing ovulation in the light-induced constant estrous-anovulatory syndrome. In: Recent advances in reproduction and regulation of fertility. Talwar P. /ed./ Elsevier, Amsterdam, pp. 37-43

Mess B., De Gaetani C., Rúzsás C., Trentini G.P. /1981/: Possible mechanisms by which pineal gland influences reproductive function. In: The pineal organ: Photobiology - biochronometry - endocrinology. Oksche A. and Pévet P. /eds./ Elsevier, Amsterdam, pp. 303-314

Mess B., Trentini G.P., Rúzsás C., De Gaetani C. /1983/: Pineal - hypothalamic interactions: possible role of the monoaminergic neuron system. In: The pineal gland and its endocrine role. Fraschini F., Axelrod J., Velo G.P. /eds./ Plenum Press, New York, pp. 361-393

Meyer D.C., Quay W.B. /1976/: Effects of continuous light and darkness and of pinealectomy, adrenalectomy and gonadectomy on uptake of H^3-serotonin by the suprachiasmatic nuclear region of male rat. Neuroendocrinology 22: 231-239

Moore R.Y. /1974/: Visual pathways and the central nervous regulation of diurnal rhythms. In: The neurosciences - Third Study Program. Schmitt F.G., Worden F.G. /eds./ M.I.T. Press, Cambridge, Mass. pp. 537-542

Moore R.Y. /1978/: Neural control of pineal function in mammals and birds. J. neural Transm. Suppl. 13: 47-58

Moore R.Y., Eichler V.B. /1976/: Central neural mechanisms in diurnal rhythm regulation and neuroendocrine responses to light. Psychoneuroendocrinology 1: 265-279

Moore R.Y., Klein D.C. /1974/: Visual pathways and the central neural control of a circadian rhythm in pineal serotonin-N-acetyltransferase activity. Brain Res. 71: 17-33

Moore R.Y., Lenn N.J. /1972/: A retino-hypothalamic projection in the rat. J. comp. Neurol. 146: 1-14

Moore R.Y., Heller A., Wurtman R.J., Axelrod J. /1967/: Visual pathway mediating pineal response to environmental light. Science 155: 220-223

Morgan W.W., Reiter R.J. /1982/: Reduced dopamine synthesis and pro - tein levels in the median eminence of blinded anosmic female rats: relation to the pineal. In: The pineal and its hormones. Reiter R.J. /ed./ Alan Liss, New York, pp. 243-252

Morgan W.W., Kamp C.W. /1983/: Extended photosensitivity of retinal dopamine neurons. Life Sci. 33: 1419-1426

Negro-Vilar A., Ojeda S.R., McCann S.M. /1979/: Catecholaminergic modulation of Luteinizing Hormone-Releasing-Hormone released by median eminence in vitro. Endocrinology 104: 1749-1756

Niles L.P., Wong Y.W., Mishura R.K. /1979/: Melatonin receptors in brain. Europ. J. Pharmacol. 55: 219-220

Nishino H., Koizumi K. /1977/: Responses of neurons in the suprachiasmatic nuclei of the hypothalamus to putative neurotransmitters. Brain Res. 120: 167-172

Nishino H., Koizumi K., McBrooks C. /1976/: The role of suprachiasmatic nuclei of the hypothalamus in the production of circadian rhythms. Brain Res. 112: 45-59

Palkovits M., Brownstein M., Saavedra J.M., Axelrod J. /1974/: Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Res. 77: 137-149

Pang S.F., Yu H.S., Suen H.C., Brown G.M. /1980/: Melatonin in the retina of rats: a diurnal rhythm. J. Endocrinol. 87: 89-95

Rance N., Barraclough C.A. /1981/: Effects of pentobarbital on hypothalamic LHRH and catecholamine turnover in proestrous rats. Proc. Soc. Exp. Biol. Med. 166: 425-431

Rance N., Wise P.M., Selmanoff M.K., Barraclough C.A. /1981/: Catecholamine turnover in discrete hypothalamic areas and related changes in median eminence Luteinizing-Hormone-Releasing-Hormone and serum gonadotrophins on proestrous and diestrous day. Endocrinology 108: 1795-1799

Reiter R.J. /1980/: The pineal and its hormones in the control of reproduction in mammals. Endocrinol. Rec. 1: 109-131

Roizen M.F., Jacobowitz D.M. /1976/: Studies on the origin of inner - vation of the noradrenergic area bordering on the nucleus raphe dorsalis. Brain Res. 101: 561-568

Rubinstein L., Sawyer C.H. /1970/: Role of catecholamines in stimulating hormones in rats. Endocrinology 86: 988-996

Rusak B. /1980/: Suprachiasmatic lesions prevent an antigonadal effect of melatonin. Biol. Reproduction 22: 148-154

Rúzsás C., De Gaetani C., Criscuolo M., Mess B., Trentini G.P. /1981/: Possible role of the midbrain serotonergic raphe nuclei in the regulation of ovulation exerted by melatonin in the rat. Neuroendocrinology Lett. 3: 331-339

Rúzsás C., De Gaetani C., Criscuolo M., Trentini G.P. /1982/: Effect of selective 5,7-dihydroxytryptamine lesions of the brain serotonergic neuron system on the ovulation maintaining effect of melatonin in rats exposed to continuous light. Neuroendocrinol. Lett. 4: 313-322

Rúzsás C., Trentini G.P., De Gaetani C., Mess B. /1983/: Involvement of the mesencephalic serotonergic raphe nuclei in the replacement of ovulation in the continuous light exposed rat. In: Integrative neurohumoral mechanisms. Endrőczi E., De Wied D., Angelucci J., Scapagnini U. /eds./ Elsevier, Amsterdam, pp. 385-392

Saavedra J.M., Brownstein M., Palkovits M., Kizer S., Axelrod J. /1974a/: Tyrosine hydroylase and dopamine-β-hydroxylase distribution in individual hypothalamic nuclei. J. Neurochem. 23: 869-871

Saavedra J.M., Palkovits M., Brownstein M., Axelrod J. /1974b/: Serotonin distribution in the rat hypothalamus and preoptic region. Brain Res. 77: 157-165

Saavedra J.M., Grobecker H., Zivin J. /1976/: Catecholamines in the raphe nuclei of the rat. Brain Res. 114: 339-345

Sakai K., Touret M., Salvert D. /1977/: Projections of the cat locus coeruleus as visualized by the horseradish peroxidase technique. Brain Res. 119: 21-41

Sallanon M., Claustrat B., Touret M. /1982/: Presence of melatonin in various cat brainstem nuclei determined by radioimmunoassay. Acta Endocrinologica 101: 161-165

Sawyer C.H., Hilliard J., Kanematsu S., Scaramuzzi R., Blake C.A. /1974/: Effects of intraventricular infusions of norepinephrine and dopamine on LH release and ovulation in the rabbit. Neuroendocrinology 15: 328-334

Steger R.W., Reiter R.J., Siler-Khodr T.M. /1984/: Interactions of pinealectomy and short photoperiod exposure on neuroendocrine axis of the male Syrian hamster. Neuroendocrinology 38: 158-163

Tima L., Flerkó B. /1974/: Ovulation induced by norepinephrine in rats made anovulatory by various experimental procedures. Neuroendocrinology 15: 346-354

Trentini G.P., Mess B., De Gaetani C., Rúzsás C. /1979/: Pineal-brain relationship. In: The pineal gland of vertebrates including man. Progr. Brain Res. 52, Kappers A.J. and Pévet P. /eds./ Elsevier, Amsterdam, pp. 341-365

Trentini G.P., Mess B., De Gaetani C., Poggioli R., Ferrari P., Di Gregorio C. /1981/: The role of melatonin and brain serotonergic system in the maintenance of rat ovarian cyclicity. In: Pineal function. Matthews C.D. and Seamark R.F. /eds./ Elsevier, Amsterdam, pp. 77-86

Turek F.W., Losee J., Olson S.H., Ellis G.B. /1983/: Pinealectomy and lesions of the suprachiasmatic nucleus affect the castration response in hamsters exposed to short photoperiods. Neuroendocrinology 36: 335-339

Ungerstedt U. /1971/: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta physiol. scand. Suppl. 367: 1-48

Van de Kar L.D., Lorens S.A. /1979/: Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. Brain Res. 162: 45-54

Zisapel N., Laudon M. /1984/: Dopamine release induced by electrical field stimulation of rat hypothalamus in vitro: inhibition by melatonin. Biochem. Biophys. Res. Communications 104: 1610-1616

EXTRAPINEAL INDOLEAMINES: FUNCTIONAL ASPECTS

The Pineal Gland
Current State of Pineal Research
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MELATONIN ACTION ON BRAIN: PRESUMPTIVE RECEPTORS AND SECOND MESSENGERS*

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INTRODUCTION

It is now generally accepted that the mammalian pineal gland is an endocrine organ that produces hormones with activity on the neuroendocrine system. One of these hormones, melatonin, is secreted as a function of time of day in all vertebrates studied insofar (Reiter, 1980; Cardinali, 1981a; Goldman and Darrow,1983). In several species, e.g. hamster, mouse, sheep, melatonin administration mimics most if not all the effects of ambient illumination on seasonal reproduction, and there is a general agreement on that melatonin acts on the brain to regulate secretory seasonality of gonadotropins and prolactin. Thus a great deal of attention has concentrated on melatonin-sensitive events at the level of the hypothalamus, a reasonable approach in view of hypothalamic role in the control of pituitary function. The following mechanisms have been advanced to explain hypothalamic actions of melatonin: binding to specific receptors (Cardinali et al. 1978,1979; Brown et al.,1979; Niles,1983); action on serotonin receptors (Wurtman and Anton-Tay,1969) interaction with benzodiazepine (BZP) binding sites (Marangos et al.,1981); inhibition of tubulin synthesis and impairment of fast axonal transport (Cardinali,1981b); reduced number of estradiol receptors (Roy and Wilson,1981); MAO inhibition (Urry and Ellis,1975); depressed synaptosomal uptake of serotonin (Cardinali,1975); depressed release of dopamine (Zisapel et al.,1983); increased GABA content (Anton-Tay,1971); increased neuropeptide release (Richardson et al.,1981); inhibition of prostaglandin E_2 (PGE_2) (Cardinali et al.,1980; Leach et al.,1982) and cyclic AMP synthesis (Vacas et al.,1981,1984a; Niles,1983); increased cyclic GMP synthesis (Vacas et al.,1981); impaired Ca^{2+} uptake (Zisapel et al., 1983; Vacas et al.,1984b). Very few of these effects, namely modified transmitter release, PG and cyclic nucleotide synthesis and Ca^{2+} uptake, have been detected in vitro at concentrations compatible with the interaction with melatonin binding sites (about 10 nM). In Syrian hamsters locally applied melatonin predominantly inhibits the elec-

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trical activity of anterior hypothalamic neurons under conditions when the exogenous administration of melatonin causes involution of the gonads (Demaine,1983).

The present article deals with the biochemical implications of some of the effects listed above particularly from the standpoints of possible intracellular second messengers for melatonin action. The interested reader is referred to recent reviews for the discussion of current concepts on melatonin physiology not covered herein (Cardinali 1981a; Goldman and Darrow,1983; Waldhauser and Wurtman,1983).

RECEPTORS

Early work supported the existence of a brain saturable melatonin uptake. The intracisternal administration of non-radioactive melatonin to rats depressed the subsequent in vivo hypothalamic uptake of labeled melatonin (Cardinali et al.,1973). However the low specific activity of the hormone available at the time when those experiments were performed precluded the physicochemical examination of the presumptive high affinity binding of melatonin in brain.

In 1978 two Laboratories published the first description of the occurrence of specific binding sites for melatonin in tissues. Cohen et al. (1978) observed cytosol binding sites in rat and hamster peripheral tissues like the ovary, uterus, testes, liver and eyes with dissociation constant (Kd)= 6 nM for high affinity sites and 550 nM for low affinities sites. No melatonin binding was detectable by the dextran-charcoal procedure employed in brain. Structural specificities of the high affinity sites suggested a requirement for the indole group and decreased affinity for indole derivatives lacking the N-acetyl group. Cardinali et al. (1978) found melatonin binding activity in crude membrane fractions of bovine medial basal hypothalamus (MBH) with Kd= 12 nM and binding site concentration of about 15 fmoles/mg prot. Binding affinities of melatonin analogues indicated that the 5-methoxy and N-acetyl groups were needed for full expression of binding to MBH membranes and that binding was abolished by the presence of a 6-hydroxy group. Binding was increased by adding Ca^{2+}, was inhibited by either Na^{+} or K^{+}, and was located mainly in the crude synaptosomal pellet. Other areas of the bovine brain also exhibited melatonin binding activity, being about 70% and 30% that of MBH in the occipital and cerebellar cortexes, whereas it was undetectable in amygdala, striatum or pons (Cardinali et al.,1979).

Differing from Cohen et al's report, Brown and co-workers (1979) did find, by using the dextran-charcoal technique, cytoplasmic melatonin binding in rat, human and bovine brain. The Kd observed varied from 9 nM (hypothalamus) to 302 nM (midbrain). As in bovine striatum (Cardinali et al.,1979)no saturable binding of melatonin to rat striatal membranes was observed (Brown et al.,1979).

The posibility that cytosol and membrane melatonin binding sites co-exist in the same tissue was first indicated by Lang and Sizonenko's observations (1980). In the liver membrane and cytosol binding sites were found, the former being Ca^{2+} dependent. Kd values were 8 nM for membrane binding sites and 20 nM for cytosol binding sites. Concentration of sites in liver membranes was about 10% that in the cytosol. Among the various tissues examined the highest membrane melatonin

receptor concentration was found in the rat hypothalamus and pituitary gland. The coexistence of brain cytosol and membrane melatonin binding sites is further supported by a recent report of Niles (1983). High levels of ^{3}H-melatonin binding were detected in brain membranes subjected to multiple washings. Binding affinity (Kd= 15-20 nM) was comparable to that reported by Vacas and Cardinali (1979) in rat brain P_2 membranes but Bmax was about 25 times greater. The pharmacological features of binding indicated that the 5-methoxy substituent is necessary for binding (Niles, 1983).

A number of reports indicated that both pineal biochemistry and morphology are affected by the exogenous injections of melatonin. If the effect of melatonin is exerted directly on pineal cells rather than indirectly via the pineal sympathetic nerves or through a second hormone acting on the pineal gland, melatonin receptors should be present in the gland. Such specific melatonin binding sites were detected by conventional radiochemical techniques in bovine pineal membranes (Vacas and Cardinali,1980) and by a semi-quantitative immunohistological procedure in rat pineal glands (Grota et al.,1981). Membrane pineal binding sites differed in a number of properties from those of MBH, including their high Kd (700 nM), ionic dependence and structural requirements; whether or not they represent pinealocyte autoreceptors remains to be defined.

Another potentially relevant site of action for melatonin is the retina. The melatonin forming enzyme hydroxyindole-O-methyl transferase is present in the mammalian retina, and the rat retina in vitro is capable of converting serotonin into melatonin (Cardinali and Rosner,1971). It is now accepted that the mammalian retina is a physiological, significant site of synthesis and locus of action of melatonin (Ralph,1981). Retinal melatonin affects photomechanical adjustment of photoreceptor cells and pigment epithelium, disc shedding and retinal dopamine metabolism (Gern et al.,1981; Besharse and Dunis, 1983; Dubocovich,1983; Ogino et al.,1983). Presumptive melatonin receptors exhibiting a Kd= 1-2 nM and a structure requirement resembling those of other brain areas have been identified in retinal tissue preparations (Gern et al.,1981).

For all the aforementioned melatonin binding sites the rigorous demonstration of their receptor nature is still lacking. However circumstantial evidence has been accumulated in favor of this view for melatonin binding sites in brain membranes. In a first experiment we tested the possibility that the affinity or number of presumptive melatonin membrane receptors in hamster and rat brains could change accordingly at times when melatonin is biologically active or inactive to cause neuroendocrine effects (Vacas and Cardinali,1979). Receptors are maximally concentrated at the late evening, a time during the diurnal cycle when exogenous melatonin is effective in inducing gonadal atrophy; conversely the receptor number is low at early morning a time when melatonin injection is uneffective in inhibiting reproductive physiology. In rat pineal the binding capacity of melatonin also increased markedly during the light phase of the day (Grota et al.,1981). Suppression of melatonin rhythmicity by pinealectomy, pineal denervation or continuous exposure to light eliminated both the antigonadal effect of single daily evening melatonin injections and the morning-evening differences in brain membrane receptor number

(Cardinali and Vacas,1981). Exposure to high melatonin levels during the dark phase of daily photoperiod may cause desensitization of the neuroendocrine system by down-regulation of receptor binding sites whereas during daytime the number of receptors increases because melatonin levels are low, and restoration of sensitivity may occur at the end of the light phase.

Another example of correlation between brain melatonin binding and biological activity is given by starvation experiments in rats. Protein deprivation in these species is accompanied by significant increases of the number of melatonin receptor sites in brain membranes without changing their Kd for the radiolabeled compound (Cardinali and Vacas,1981) and it has been shown an increase in the sensitivity of the reproductive system to exogenous and presumably endogenous pineal melatonin in underfed rats (Reiter,1980).

It should be noted that a reproducible and detailed characterization of melatonin binding sites has been hampered by the poor quality and instability of the commercially available ligand. In our hands the initial observations on melatonin receptors in bovine, rat and hamster brain membranes carried out by employing (2-aminoethyl-2-^{3}H) melatonin (New England Nuclear) between 1977 and 1980 were followed by negative results obtained with subsequent batches of the compound purchased from the same commercial source. Last year the supplier discontinued the production of this radioligand and began to provide a new ^{3}H-melatonin labeled in the methoxy group. In our experience this new melatonin is suitable to associate the receptors only for a limited interval after delivery, and our attempts to stabilize such a binding have not yet been successful. Obviously further studies are needed to establish whether brain melatonin binding sites (cytoplasmic and membrane) are truly physiological receptors for the hormone.

An interesting observation about a possible molecular locus for melatonin action in brain cells has been the description that melatonin and its brain metabolite N-acetyl-5-methoxy kynurenamine interact at concentrations greater than 10 uM with BZP binding sites in vitro (Marangos et al.,1981). These sites are considered the pharmacological receptors for the anticonvulsivant, anxiolytic, sleep-promoting and muscle relaxant properties of BZP (Haefely et al.,1983). One of the mechanisms by which BZP act on the brain is the enhancement of GABA neurotransmission, and there is experimental evidence suggesting that the BZP binding site is a part of a supramolecular complex formed also by the GABA receptor, the Cl^{-} channel and a barbiturate recognition site. Additionally BZP like several other neuroactive drugs could affect synaptic transmission via interaction with the synaptic Ca^{2+}-calmodulin system (De Lorenzo,1981).

Melatonin shares muscle-relaxant and anticonvulsivant properties with BZP (Sugden,1983). Moreover melatonin may increase GABA neurotransmission as suggested by the elevation of brain GABA levels after systemic injection (Anton-Tay,1971), and as discussed below it can interfere with Ca^{2+} influx at isolated nerve terminals. This prompted us to assess whether pineal removal or melatonin treatment could affect BZP receptor density in rat cerebral cortex, a region of the brain that exhibits high concentration of BZP binding sites.

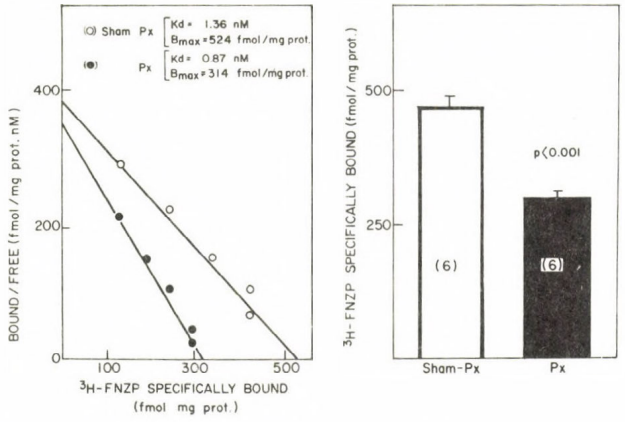

Fig. 1 Effect of pinealectomy (Px) or sham-operation performed 14 days earlier on BZP binding sites in rat cerebral cortex assayed by the binding of ^{3}H-flunitrazepam (FNZP). Data are shown as Scatchard plots (left panel) or as single-point binding assays carried out at 6 nM ^{3}H-FNZP (means + SEM,n). Slopes and intercepts in saturation isotherm experiments were calculated by regression analysis and analyzed statistically by means of an analysis of covariance ($p < 0.01$ for differences in Bmax values). Single-point binding assays of individual brains were statistically analyzed by a Student's *t* test.

As shown in Fig.1 pinealectomy of rats resulted in significant depression of BZP receptor sites of cerebral cortex 14 days later without affecting their affinity significantly. These observations should be analyzed within the context of the homeostatic influence of the pineal gland on CNS excitability (Quay,1975; Romijn,1978). Removal of the pineal gland in rabbits changes the electrical activity of dorsal hippocampal neurons towards an epileptiform electroencephalographic pattern (Bindoni and Rizzo,1975). In pinealectomized rats the electrical recording of cerebral cortex exhibited intermitent paroxysmal outbursts of seizure-like discharges (Nir et al.,1969) and in parathyroidectomized rats a subsequent pinealectomy induced violent and often fatal clonic-tonic type convulsions (Reiter et al.,1973). Providing that an endogenous agonist ligand does exist for BZP receptors in brain (Haefely et al.,1983), the convulsive prone state or increased paradoxical sleep (Romijn,1978) of pinealectomized rats could reflect an impaired BZP receptor-GABA interaction and consequently an impaired GABA neurotransmission after pineal removal.

Melatonin treatment protects against pinealectomy-induced seizures (Rudeen et al.,1980; Sugden,1983). It also improved electroencephalographic activity of patients with temporal lobe epilepsia (Anton-Tay,1971), diminished light-induced seizures in baboons (Brailowsky,1976) and reduced focal epileptic activity of primary sensory areas in cats (Fariello and Bubenik,1976). As shown in Fig.2 a single injection of 0.8 mg/kg of melatonin restored 3 h later the depressed BZP receptor sites in pinealectomized rats. Daily melatonin

injections during 5 days were needed to affect BZP binding sites in intact rats (data not shown). Although melatonin added in vitro to cerebral cortex membranes competed for BZP binding at concentrations 10 uM or greater (Marangos et al.,1981) such concentrations are presumably not achieved in brain at the doses employed by us. Pinealectomy and melatonin-induced changes of BZP binding sites reported herein may thus result from a modulatory indirect effect rather than from a direct effect of the hormone on the binding sites. Our results suggest the possibility that pineal-related changes in brain excitability could be linked to melatonin effect on BZP receptor sites.

CYCLIC NUCLEOTIDES

Melatonin can like many other neurohormones or transmitters,modulate the activity of adenylate cyclase in brain. The first evidence about the involvement of cyclic AMP in any biologic effect of melatonin was provided by Abe et al. (1969) in the amphibian skin. Melatonin added in vitro not only inhibited frog skin's darkening response to MSH but also inhibited the rise in cyclic AMP; melatonin differed from norepinephrine (NE) in that its effects could not be blocked by α-adrenoceptor antagonists.

That the pineal gland and melatonin may be linked to functional changes in cyclic AMP synthesis at brain target sites in vivo was

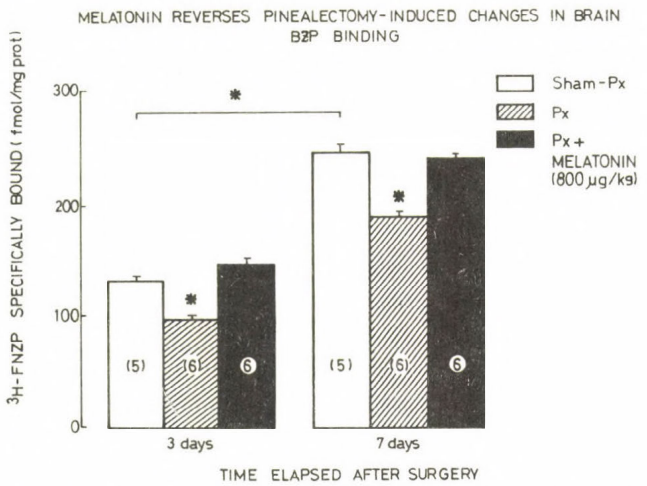

Fig. 2 Effect of melatonin (800 ug/Kg, 3 h earlier) on pinealectomy (Px)-induced decrease of BZP binding in rat cerebral cortex, 3 or 7 days after surgery. Shown are the means + SEM of individual brains assayed at 6 nM ^{3}H-FNZP. Bmax of Px rats differed from the other 2 groups at either time interval ($p < 0.05$, analysis of variance). No statistically differences in binding affinity of the 3 experimental groups were detectable (data not shown).

suggested by the studies of Morguenstern et al.(1983,1984). Pineal ablation increased significantly cyclic AMP synthesis by 52% in rat MBH 3 days after surgery whereas 2 doses of 100 ug of melatonin given to rats 17 and 2 h before sacrifice decreased hypothalamic cyclic AMP by 33%. In rat striatal and hypothalamic slices NE increases cyclic AMP by interacting with α- and β-adrenoceptors (Partington et al.,1980; Leblanc and Cianarello,1984). After superior cervical ganglionectomy a portion of hypothalamic noradrenergic terminals undergo degeneration as revealed by the decreased NE content in median eminence of peripherally denervated rats (Chiocchio et al, 1984). These changes are accompanied by an augmented in situ cyclic AMP synthesis and binding to intracellular receptors as well as by modified LH, FSH and TSH release (Cardinali et al.,1982; Chiocchio et al.,1984). Ganglia and pineal ablation have additive effects as revealed by the further increase of MBH cyclic AMP after the combined surgical procedure. Since within 16-24 h after ganglionectomy melatonin synthesis rises several-fold as a consequence of pineal nerve degeneration (Vacas et al.,1982) and inasmuch as melatonin depresses MBH cyclic AMP synthesis (Morguenstern et al.,1983) the additive effects of ganglionectomy and pinealectomy are best explained in terms of the removal of a negative influence on MBH cyclic AMP.

A direct assessment of melatonin effect on brain cyclic AMP synthesis was made in vitro by Vacas et al. (1981). Rat MBH explants incubated with melatonin exhibited depressed cyclic AMP levels at methoxyindole concentrations 10 nM or greater (Fig.3), 5-Methoxytryptophol was more potent than melatonin in inhibiting cyclic AMP synthesis whereas the melatonin inactive metabolite 6-hydroxymelatonin was essentially devoid of activity at physiological concentrations. Recently Niles (1983) reported that melatonin inhibits adenylate cyclase in rat hypothalamic and pineal homogenates by producing a GTP dependent inhibition of enzyme activity.

Since it was impossible from the above cited experiments to conclude about the brain cells (glial or neurons) affected by melatonin Vacas et al. (1984a) undertook a series of experiments employing astroglial cell cultures identified according to morphological and immunochemical criteria. Primary rat brain cultures are considered suitable models of untransformed astroglial cells, and are used increasingly for neurobiological studies. In the astroglial subcultures identified by immunolabelling of glial fibrillary acid protein, melatonin although not altering cyclic AMP content by itself, brought about at submicromolar concentrations an impaired β-adrenoceptor-mediated increase of cyclic AMP levels. This effect of melatonin was shared by 5-methoxytryptophol that is a strong competitor of melatonin for its putative MBH membrane receptors (Cardinali et al.,1979; Niles,1983), and by the potent synthetic melatonin analogue 6-chloromelatonin (Fig.4). Serotonin exhibited considerably less activity and 6-hydroxymelatonin did not affect cyclic AMP levels.

Our findings are in agreement with the view that at least a part of melatonin effect on cyclic AMP synthesis (Vacas et al.,1981) or adenylate cyclase (Niles,1983) of brain explants or homogenates takes place in astrocytes. As first suggested by Quay (1970) the glial cells may be one of the sites of action for pineal melatonin.

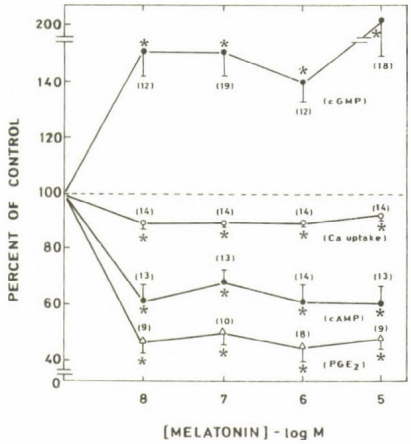

Fig. 3 In vitro effect of melatonin on cyclic AMP, cyclic GMP and PGE_2 synthesis by the rat MBH, and on $^{45}Ca^{2+}$ uptake by rat brain synaptosomes. Shown are the means ± S.E.M. (n). Asterisks designate significant changes as compared to controls ($p < 0.01$, analysis of variance). For experimental details see Cardinali et al. (1980) and Vacas et al. (1981; 1984b).

Melatonin could act theoretically at the interaction of guanine nucleotides with the guanine nucleotide regulatory protein (or "G" unit) which is known to mediate the effect of β-adrenoceptor agonist on adenylate cyclase. This is supported by the recently described GTP-dependent inhibition of enzyme activity by melatonin in brain homogenates (Niles,1983). Besides, melatonin could impair NE-evoked cyclic AMP increase by the inhibition of PGE_2 synthesis discussed below. In any event it seems safe to conclude that melatonin resembling other hormones, autacoids and transmitters that act on receptors located on the cell surface, depresses the adenylate cyclase in brain target cells.

Another brain cyclic nucleotide affected by melatonin is cyclic GMP. The injection of 1 ug of melatonin in the cisterna magna of rabbits elicited a rise in CSF cyclic GMP levels (Rudman,1976). In vitro melatonin at 10 nM concentration increased significantly cyclic GMP accumulation by incubated rat MBH explants (Fig.3). For this effect 5-methoxy-tryptophol was about 1000 times less potent than melatonin, and 6-hydroxymelatonin was inactive (Vacas et al.,1981). In view that melatonin enhances guanylate cyclase activity in rat anterior pituitary, thyroid, testis, ovary, liver and intestine (Vesely,1981) the possibility should be considered that a general effect of the hormone is to increase cellular levels of cyclic GMP. There is no information on melatonin effect on cyclic GMP synthesis in astroglial cultures.

PROSTAGLANDINS

Any neuroendocrine transducing event implies the conversion of a neural signal arriving at a group of cells to a hormonal signal that is released to the extracellular space. In order for the cells to respond to external inputs they must have mechanisms both for detecting and for translating the incoming information. One is given by adenylate cyclase, as discussed above. Another lies in the lipid portions of the membrane, which in addition to be the matrix containing the transductive proteins, are the site of origin of a number of intracellularly active metabolites related to the hydrolysis of phosphatidylinositol and to the increase of cellular Ca^{2+} levels. One of these mechanisms is subserved by PGs, the best characterized arachidonic acid metabolites. Arachidonic acid metabolism gives rise to a variety of active compounds including PGs, thromboxanes, leukotrienes and hydroxy and hydroperoxy acids (Wolfe, 1982). The whole spectrum of oxygenated arachidonic acid derivatives, or the eicosanoid system, is characterized by the following functional features: (i) easy activation of these pathways in response to a number of stimuli which generally alter the rate of synthesis through the increased release of arachidonic acid from its phospholipidic repositories; (ii) the basically local action of eicosanoids as intracellular messengers and/or autacoids due to the ubiquitous synthesis; (iii) interaction with nearby receptors and rapid degradation; (iv) the presence of multiple enzymatic sites of feedback regulation in the whole arachidonic acid cascade. In neuroendocrine junctions like the MBH or the pineal gland PGs may subserve the role of second messengers within the cells; in other cases they diffuse away from the cell and act as local hor-

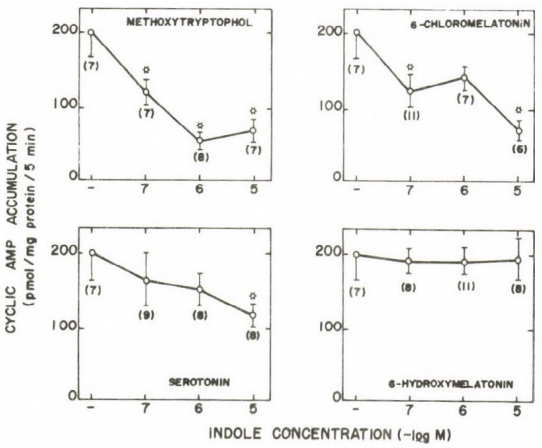

Fig. 4 Effect of several indoles on isoproterenol-induced cyclic AMP accumulation in rat astroglial cultures. Shown are the means ± SEM (n). Asterisks designate significant differences as compared to cultures incubated in the absence of indole ($p < 0.05$ analysis of variance). For experimental details see Vacas et al. (1984a).

mones to modulate the activity of surrounding cells (Cardinali and Ritta, 1983).

The best characterized effect of PGE_2 on the neuroendocrine hypothalamus is that on LHRH release; PGE_2 decreased the hypothalamic content of LHRH and induced LHRH release into the portal blood (Eskay et al., 1975); moreover the rise of serum LH elicited by PGE_2 was prevented by the simultaneous administration of LHRH antiserum (Chobsieng et al., 1975). Fragments of median eminence or MBH incubated with different concentrations of NE resulted in dose-related increases of PGE_2 through interaction with α-adrenoceptors and PGE_2 stimulates LHRH release from MBH in vitro presumably by acting on LHRH-secreting cells (Ojeda et al., 1981). The PG-synthesis inhibitor indomethacin prevented LH release and ovulation when injected in vivo and NE-induced LHRH release in rat MBH explants in vitro (Ojeda et al, 1981).

Based on the structural similarities between melatonin and indomethacin (N-p[chlorobenzol]-2-methyl-5-methoxyindole-3-acetic acid) Leach and Thornburn (1980) and we (Cardinali et al., 1980; Gimeno et al., 1980) put forth independently the hypothesis that the pineal hormone could depress PG synthesis at the level of its target cells. Indeed melatonin and indomethacin share several biochemical and pharmacological properties. Both have common antigenic properties, inhibit the post-castration and steroid-induced rise of LH at a hypothalamic site, and affect central and peripheral phenomena linked to PG production (Cardinali, 1981a). When the effects of submicromolar concentrations of melatonin on rat MBH PGE_2 synthesis in vitro were assessed a depression of the spontaneous (Fig.3) and NE-induced PGE_2 release was observed at melatonin concentrations of 10 nM or higher (Cardinali et al., 1980). Keeping in pace with this observation Leach et al (1982) reported that 400 ug of melatonin injected into the CSF prevented both ovulation as well as the increase of PGE_2 in CSF following the stimulation of uterine cervix in mature rabbits. Thromboxane release from platelets and platelet aggregation (a thromboxane-dependent phenomenon) are also inhibited by melatonin (Leach and Thornburn, 1980; Gimeno et al, 1981). Therefore the possibility arises that at least some of melatonin effects on brain cells can be attributed to inhibition of cyclooxygenase activity.

CALCIUM

Ca^{2+} plays a significant role in the function of the nervous system. The release of neurotransmitters is dependent on ambient Ca^{2+} concentration through an effect unrelated to the presynaptic action potential but directly linked to the entry of Ca^{2+} into the presynaptic terminal (De Lorenzo, 1981). In view that melatonin (0.1 nM-1 uM) inhibited both the electrically-induced and K^+-stimulated release of dopamine from the rat hypothalamus in vitro, Zisapel and Laudon (1983) examined Ca^{2+}-dependency of dopamine release evoked by electrical field stimulation of rat hypothalamic tissue. These authors found that nanomolar concentrations of melatonin inhibited Ca^{2+}-dependent dopamine release, as well as reduced tissue uptake of $^{45}Ca^{2+}$ during stimulation caused by either an electrical field or elevated K^+ concentration. Since the Ca^{2+} ionophore A 23187 blunted the inhibition by melatonin of dopamine release and Ca^{2+} uptake

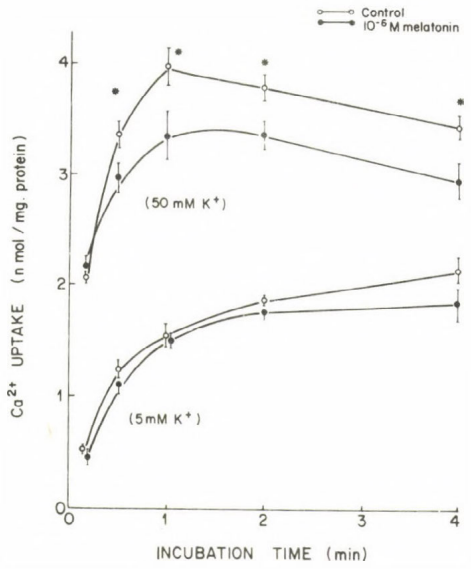

Fig. 5 Effect of melatonin on $^{45}Ca^{2+}$ uptake by rat brain synaptosomes determined at 37 °C in an assay system containing 5 mM KCl or 50 mM KCl. Shown are the means ± SEM (n= 6 in each group). Asterisks designate significant differences between melatonin and control groups ($p < 0.05$, Student's t test). For experimental details see Vacas et al. (1984b).

Zisapel and Laudon (1983) concluded that the inhibitory effect of melatonin on transmitter release depended on the reduction of Ca^{2+} efluxes into the presynaptic terminals. However no data were provided on the effect of melatonin on basal, unstimulated $^{45}Ca^{2+}$ uptake in hypothalamic slices and other possible mechanisms as a hyperpolarization of the transmitter-releasing neuron rendering it less susceptible to action potentials should be also considered. Therefore we set up a series of experiments aiming to assess whether in brain synaptosomes depolarized by high K^+ buffer the resultant increase of Ca^{2+} influx as well as the basal uptake were affected by melatonin and its analogues (Vacas et al.,1984b).

In synaptosomes voltage-dependent Ca^{2+} channels are activated during high K^+ stimulation allowing the diffusion of Ca^{2+} into the intracellular space. To evoke neurotransmitter release intracellular Ca^{2+} concentration must increase transiently about two orders of magnitude to a peak of 1-10 uM (Rubin,1982). Subsequently intracellular organelles like the endoplasmic reticulum or the mitochondria provide the buffering system to sequester free Ca^{2+} thus restoring the resting state. The time course of $^{45}Ca^{2+}$ uptake into rat brain synaptosomes indicated that after a rapid influx during the first 30s Ca^{2+} uptake reached equilibrium at about 1 min (Fig.5). Melatonin up to 1 uM failed to affect $^{45}Ca^{2+}$ uptake in low K^+ medium but decreased significantly the uptake stimulated by 50 mM K. Other methoxyindoles, i.e. 5-methoxytryptophol, 5-methoxytryptamine and 6-chloromelatonin

(10 nM- 1 uM) also depressed significantly the K^+-stimulated increase of synaptosome Ca uptake, in some cases even in a more active way than melatonin (Vacas et al.,1984b). Serotonin whose indole ring is devoid of a methoxy moeity was essentially inactive on the parameter examined. Therefore our results and those of Zisapel and Laudon (1983) indicate that pineal methoxyindoles impair Ca^{2+} entry into depolarized nerve endings, by affecting directly or indirectly the Ca channel. In the brain the calmodulin-Ca^{2+} complex mediates most of the effects of Ca^{2+} on neurotransmission including endogenous synaptic Ca^{2+}- calmodulin protein kinase activation, neurotransmitter release, and synaptic vesicle-synaptic membrane interaction (De Lorenzo,1981). Neuroactive drugs like trifluoroperazine, a phenotiazine that inactivate calmodulin or diazepam, a BZP that antagonizes the effect of the Ca^{2+}-calmodulin complex on protein kinase,may affect neurotransmission partly by interference with the Ca^{2+}-calmodulin system (De Lorenzo,1981). In view of the above reported effects of melatonin on brain BZP receptor activity and $^{45}Ca^{2+}$ uptake it is tempting to postulate that the pineal hormone interferes both with Ca^{2+} influx and with Ca^{2+} mediated phenomena at its target cells in brain. Undoubtely some of these phenomena may include cyclic nucleotide and PGE_2 synthesis (Rubin,1982).

PHYSIOLOGICAL IMPLICATIONS OF SECOND MESSENGERS' CHANGES

At least two questions arise from the foregoing discussion: (i) might the melatonin binding sites and changes in second messengers above reported be related in a causal way to the in vitro neurochemical effects of melatonin ?; (ii) are these effects linked to the neuroendocrine activity of melatonin on endocrine seasonality ?. Although some speculations on the first point can be entertained, the answer to the second question is largely unknown.

Melatonin appears to act on neurons or glial cells to set the "tonus" to receive another message, like a transmitter released at the synaptic cleft or a circulating hormone attaining the cells from the blood stream. That melatonin acts on its own specific recognition sites in cell membranes and cytoplasm and not on serotonin receptors is now supported by radioligand binding studies, neurochemical data and neuropharmacological experiments (e.g. see Sugden,1983). A feasible primary post-receptor event in melatonin action can be the impairment of Ca^{2+} influx. Indeed Ca^{2+} and calmodulin are known to regulate several synaptic processes in isolated and intact brain preparations, and through the activation of synaptic Ca^{2+}-calmodulin protein kinase may affect membrane function (De Lorenzo,1981). Thus the reduced Ca^{2+} uptake elicited by melatonin could modify cyclic nucleotide and PG production by affecting cell membrane enzymes like adenylate and guanylate cyclases, phospholipases or cyclooxygenase.

Another possibility is that the primary post-receptor event triggered by melatonin is modified cyclic nucleotide synthesis,melatonin modulating Ca^{2+} channel as a consequence of decreased levels of cyclic AMP or increased levels of cyclic GMP in target cells. Impairment of cyclic AMP synthesis could be exerted at the interaction of guanine nucleotides with the guanine nucleotide regulatory protein which is known to mediate the effects of β-adrenoceptor agonists on adenylate cyclase. Recent experimental data support this

view (Niles,1983). Melatonin could also impair NE-evoked cyclic AMP increase by the primary inhibition of PGE_2 synthesis, since PGs are potent stimulators of cyclic AMP production in glial cultures and brain slices (Partington et al.,1980; Wolfe,1982). It is not known whether melatonin impairs PGE_2 synthesis in glial cells.

Summarizing from the amount of data on the subject and based on what is known from other systems it can be postulated that melatonin-induced post-receptor events include inhibition of Ca^{2+} influx and subsequent modification of cyclic nucleotide and PGE_2 synthesis. Through this sequence of intracellular phenomena melatonin can affect target neuron and glial cells.

The physiological correlates of melatonin-receptor interaction and second messengers' changes with the neuroendocrine activity of the hormone deserve considerable more exploration. Melatonin effects on the neuroendocrine system tend to be modulatory rather than primary, that is, the hormone changes the timing or amplitude of the biologic response rather than given a primary generative signal for the response. Melatonin receptor changes after manipulation of endogenous melatonin secretion (Vacas and Cardinali,1979; Cardinali and Vacas,1981) and the presumably modified Ca^{2+} fluxes and cyclic nucleotide and PG synthesis arising in this situation could well explain the phenomenon of tolerance after sustained melatonin administration and the photorefractory phase in mammalian seasonal reproduction (Reiter,1980; Goldman and Darrow,1983).

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REFERENCES

Abe,K., Robison,G.A., Liddle,G.W., Butcher,R.W., Nicholson,W.E., Baird,C.E. (1969) Role of cyclic AMP in mediating the effect of MSH, norepinephrine and melatonin on frog skin color. Endocrinology 85: 674-682.

Anton-Tay,F. (1971) Pineal-brain relationships. In The Pineal Gland. Wolstenholme,G.E.W., Knight,J. (eds.), Churchill Livingstone, Lonson, pp. 213-227.

Beshares,J.C., Dunis,D.A. (1983) Methoxyindoles and photoreceptor metabolism: activation of rod shedding. Science NY 219: 1341-1343.

Bindoni,M., Rizzo,R. (1965) Hippocampal evoked potentials and convulsive activity after electrolytic lesions of the pineal body in chronic experiments on rabbits. Archs.Sci.Biol. 49: 223-233.

Brailowsky,S. (1976) Effects of melatonin on the photosensitive epilepsy of the baboon, Papio papio, Electroenceph.Clin.Neurophysiol. 41: 314-319.

Cardinali,D.P. (1975) Changes in hypothalamic neurotransmitter uptake following pinealectomy, superior cervical ganglionectomy or melatonin administration to rats. Neuroendocrinology 19: 91-95.

Cardinali,D.P. (1981a) Melatonin. A mammalian pineal hormone. Endocrine Rev. 2: 327-346.

Cardinali,D.P. (1981b) Molecular biology of melatonin. Assessment of the "microtubule hypothesis of melatonin action". Adv. Biosci. 29: 247-256.

Cardinali,D.P., Ritta,M.N. (1983) The role of prostaglandins in neuroendocrine junctions: studies in the pineal gland and the hypothalamus. Neuroendocrinology 36: 152-160.

Cardinali,D.P., Rosner,J.M. (1971) Metabolism of serotonin by the rat retina in vitro. J.Neurochem. 18: 1769-1770.

Cardinali,D.P., Vacas,M.I. (1981) Molecular endocrinology of melatonin: receptor sites in brain and peripheral organs. Adv. Biosci. 29: 237-246.

Cardinali,D.P., Hyyppä,M.T., Wurtman,R.J. (1973) Fate of intracisternally injected melatonin in the rat brain. Neuroendocrinology 12: 30-40.

Cardinali,D.P., Vacas,M.I., Boyer,E.E. (1978) High affinity binding of melatonin in bovine medial basal hypothalamus. IRCS Med.Sci. 6: 357.

Cardinali,D.P., Vacas,M.I., Boyer,E.E. (1979) Specific binding of melatonin in bovine brain. Endocrinology 105: 437-441.

Cardinali,D.P., Ritta,M.N., Fuentes,A.M., Gimeno,M.F., Gimeno, A.L. (1980) Prostaglandin E release by rat medial basal hypothalamus in vitro. Inhibition by melatonin at submicromolar amounts. Europ.J.Pharmacol. 67: 151-153.

Cardinali,D.P., Pisarev,M.A., Barontini,M., Juvenal,G.J., Boado, R.J., Vacas,M.I. (1982) Efferent neuroendocrine pathways of sympathetic superior cervical ganglia. Early depression of the pituitary-thyroid axis after ganglionectomy. Neuroendocrinology 35: 248-254.

Chiocchio,S.R., Cardinali,D.P., Vacas,M.I., Tramezzani J.H. (1984) Acute superior cervical ganglionectomy depresses the postcastration rise of gonadotropins in male rats. Brain Res., in press.

Chobsieng,P., Naor,Z., Koch,Y., Zor,U., Lindner,H.R. (1975) Stimulatory effect of prostaglandin E_2 on LH release in the rat. Evidence for a hypothalamic site of action. Neuroendocrinology 17: 12-17.

Cohen,M., Roselle,D., Chabner,B., Schmidt,T.J., Lippman,M. (1978) Evidence for a cytoplasmic melatonin receptor. Nature 274: 894-895.

De Lorenzo,R.J. (1981) The calmodulin hypothesis of neurotransmission. Cell Calcium 2: 365-385.

Demaine,C. (1983) Modification of hypothalamic electrical activity by pineal indoles. In The Pineal Gland and Its Endocrine Role. Axelrod,J., Fraschini,F., Velo,G.P. (eds.), Plenum, New York, pp. 417-436.

Dubocovich,M.D. (1983) Melatonin in a potent modulator of dopamine release in the retina. Nature 306: 782-784.

Eskay,R.L., Warberg,J., Mical,R.S., Porter,J.C. (1975) Prostaglandin E_2-induced release of LHRH into hypophysial portal blood. Endocrinology 97: 816-824.

Fariello,R., Bubenik,G. (1976) Melatonin-induced changes in the sensory activation of acute epileptic foci. Neurosci.Lett. 3: 151-155.

Gern,W.A., Gorell,T.A., Owens,D.W. (1981) Melatonin and pigment cell rhythmicity. Adv.Biosci. 29: 223-233.

Gimeno,M.F., Landa,A., Speziale,N.B., Cardinali,D.P., Gimeno,A. L. (1980) Melatonin blocks in vitro generation of prostaglandin by the uterus and hypothalamus. Europ.J.Pharmacol. 62: 309-317.

Gimeno,M.F., Ritta,M.N., Bonacossa,A., Lazzari,M., Gimeno,A.L., Cardinali,D.P. (1981) Inhibition by melatonin of prostaglandin synthesis in hypothalamus, uterus, and platelets. Adv.Biosci. 29: 147-150.

Goldman,B.D., Darrow,J.M. (1983) The pineal gland and mammalian photoperiodism. Neuroendocrinology 37: 386-396.

Grota,L., Holloway,W., Brown,G. (1981) Melatonin receptors in pineal. Adv.Biosci. 29: 257-261.

Haefely,W., Polc,P., Pieri,L., Schaffner,R., Laurent,J.-P. (1983) Neuropharmacology of benzodiazepines: synaptic mechanisms and neural basis of action. In The Benzodiazepines: From Molecular Biology to Clinical Practice, Costa,E. (ed.) Raven, New York, pp 21-65.

Lang,U., Sizonenko,P.C. (1980) Tissue and cellular location of melatonin receptors. Internat.Symposium on Melatonin, Bremen, F.R.G., Abs. nº 143.

Leach,C.M., Thornburn,G.D. (1980) A comparison of the inhibitory effects of melatonin and indomethacin on platelet aggregation and thromboxane release, Prostaglandins 20: 51-56.

Leach,C.M., Reynoldson,J.A., Thornburn,G.D. (1982) Release of E prostaglandins into the cerebrospinal fluid and its inhibition by melatonin after cervical stimulation in the rabbit. Endocrinology 105: 437-441.

Leblanc,G.G., Cianarello,R.D. (1984) Noradrenergic potentiation of neurotransmitter-stimulated cAMP production in rat striatal slices, Brain Res. 293: 57-65.

Marangos,P.J., Patel,J., Hirata,F., Sondhein,D., Paul,S.M., Skolnick,P., Goodwing,F.K. (1981) Inhibition of diazepam binding by tryptophan derivatives including melatonin and its brain metabolite N-acetyl-5-methoxykynurenamine, Life Sci. 29: 256-267.

Morguenstern,E.A., Vacas,M.I., Keller Sarmiento,M.I., Cardinali, D.P. (1983) Melatonin injection depresses and pinealectomy increases cyclic AMP levels in rat medial basal hypothalamus. Com. Biol. 1: 347-350.

Morguenstern,E.A., Vacas,M.I., Keller Sarmiento,M.I., Cardinali, D.P. (1984) Pineal-related changes in cyclic AMP levels of rat medial basal hypothalamus. Experientia 40: 223-224.

Niles,L.P. (1983) Melatonin binding in brain membranes and effects on adenylate cyclase. 13th Ann.Meeting Society for Neurosciences. Boston, Abs nº 212.4.

Niles,L.P., Wong,Y.W., Mishra,R.K., Brown,G.M. (1979) Melatonin receptors in brain. Europ.J.Pharmacol. 55: 219-220.

Nir,I., Behroozi,K., Assael,M., Ivriani,I., Sulman,F.G. (1968) Changes in the electrical activity of the brain following pinealectomy, Neuroendocrinology 4: 122-127.

Ogino,N., Matsumura,M., Shirakawa,H., Tsukahara,I. (1983) Phagocytic activity of cultured retinal pigment epithelial cells from chick embryo: inhibition by melatonin and cyclic AMP, and its reversal by taurine and cyclic GMP. Ophtalmic Res. 15: 72-89.

Ojeda,S.R., Negro-Vilar,A., McCann,S.M. (1981) Role of prostaglandins in the control of pituitary hormone secretion. Prog. Clin.Biol.Res. 74: 229-247.

Partington,C.R., Edwards,M.W., Daly,J.W. (1980) Regulation of cyclic AMP formation in brain tissue by α-adrenergic receptors: requisite intermediacy of prostaglandins of the E series. Proc. natn.Acad.Sci. USA 77: 3024-3028.

Quay,W.B. (1970) Endocrine effects of the mammalian pineal. Am.Zoologist 10: 237-249.

Ralph,C.L. (1981) Melatonin production by extra-pineal tissues. Adv.Biosci. 29: 35-46.

Reiter,R.J. (1980) The pineal and its hormones in the control of reproduction. Endocrine Rev. 1: 109-131.

Reiter,R.J., Blask,D.E., Talbot,J.A., Barnett,M.P. (1973) Nature and time course of seizures associated with surgical removal of the pineal gland from parathyroidectomized rats. Exp.Neurol. 38: 386-397.

Richardson,S.B., Hollander, Prasad,J.A., Hirooka,T. (1981) Somatostatin release from rat hypothalamus in vitro; effects of melatonin and serotonin. Endocrinology 109: 602-606.

Romijn,H. (1978) The pineal, a tranquillizing organ? Life Sci. 23: 2257-2274.

Roy,E.J., Wilson,M.A. (1981) Diurnal rhythm in cytoplasmic estrogen receptors in the rat brain in the absence of circulating estrogen. Science, N.Y. 213: 1525-1526.

Rubin,R.P. (1982) Calcium-phospholipid interactions in secretory cells: a new perspective on stimulus-secretory coupling. Fed. Proc. 41: 2181-2187.

Rudeen,P.K., Philo,R.C., Symmes,S.K. (1980) Antiepileptic effects of melatonin in the pinealectomized mongolian gerbil. Epilepsia 21: 149-154.

Rudman,D. (1976) Injection of melatonin into cisterna magna increases concentration of 3',5'-cyclic guanosine monophosphate in cerebrospinal fluid. Neuroendocrinology 20: 235-242.

Sugden,D. (1983) Psychopharmacological effects of melatonin in mouse and rat. J.Pharmacol.Exp.Ther. 227: 587-591.

Urry,R.L., Ellis,L.C. (1975) Monoamine oxidase activity of the hypothalamus and pituitary: alterations after pinealectomy, changes in photoperiod or addition of melatonin in vitro. Experientia 31: 891-892.

Vacas,M.I., Cardinali,D.P. (1979) Diurnal changes in melatonin binding sites of hamster and rat brains. Correlation with neuroendocrine responsiveness to melatonin. Neurosci.Lett. 15: 259-263.

Vacas,M.I., Cardinali,D.P. (1980) Binding sites for melatonin in bovine pineal gland. Hormone Res. 13: 121-130.

Vacas.M.I., Keller Sarmiento,M.I., Cardinali,D.P. (1981) Melatonin increases cGMP and decreases cAMP levels in rat medial basal hypothalamus in vitro. Brain Res. 225: 207-211.

Vacas,M.I., Keller Sarmiento,M.I., Pereyra,E.N., Cardinali,D.P. (1982) Early changes in cAMP and melatonin levels of rat pineal gland after superior cervical ganglionectomy. Neuroendocrinology Lett. 4: 267-271.

Vacas,M.I., Berría,M.I., Cardinali,D.P., Lascano,E.F. (1984a) Melatonin inhibits β-adrenoceptor-stimulated cyclic AMP accumulation in rat astroglial cell cultures. Neuroendocrinology 38: 176-181.

Vacas,M.I., Keller Sarmiento,M.I., Cardinali,D.P. (1984b)Pineal methoxyindoles depress calcium uptake by rat brain synaptosomes. Brain Res. 294: 166-168.

Vesely,D.L. (1981) Melatonin enhances guanylate cyclase activity in a variety of tissues. Molec.Cell Biochem. 35: 55-58.

Waldhauser,F., Wurtman,R.J. (1983) The secretion and action of melatonin. In Biochemical Actions of Hormones, Vol.10, Litwack, G. (ed.), Academic Press, New York, pp. 187-225.

Wolfe,L.S. (1982) Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. J.Neurochem. 38: 1-14.

Wurtman,R.J., Anton-Tay,F. (1969) The mammalian pineal as a neuroendocrine transducer. Recent Prog.Horm.Res. 25: 493-522.

Zisapel,N., Laudon,M. (1983) Inhibition by melatonin of dopamine release from rat hypothalamus: regulation of calcium entry. Brain Res. 272: 378-381.

Zisapel,N., Egozi,Y., Laudon,M. (1983) Inhibition by melatonin of dopamine release from rat hypothalamus in vitro: variations with sex and the estrous cycle. Neuroendocrinology 37: 41-48.

The Pineal Gland
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EXTRAPINEAL MELATONIN AND N-ACETYLSEROTONIN

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INTRODUCTION

Historically, the indolealkylamine which has received most attention is serotonin. Its presence and actions have been identified in various tissues including platelets (Gaddum 1936; Page 1954), gastrointestinal tract (Erspamer 1966) and central nervous system (Twarog and Page 1953; Osborne 1982).

Melatonin (Lerner et al, 1958) and N-acetylserotonin (Brownstein et al, 1973) were first identified in the pineal gland. In the pineal gland serotonin is converted to N-acetylserotonin by the enzyme N-acetyltransferase (Weissbach et al, 1960). N-acetylserotonin is subsequently converted to melatonin by hydroxyindole-O-methyltransferase (Axelrod and Weissbach 1961). The rate limiting step in this pathway is N-acetyltransferase. This enzyme shows a marked circadian rhythm with highest activity during darkness, which is regulated by sympathetic input to the pineal gland acting on beta adrenergic receptors on the pinealocytes (Klein and Weller 1970). Although N-acetyltransferase (NATase) and hydroxyindole-O-methyltransferase (HIOMT) activites are highest in the pineal gland they are not confined to that tissue (Ellison et al, 1972; Paul et al, 1974; Cardinali and Rosner 1971). Hence synthesis of N-acetylserotonin and melatonin may take place outside of the pineal.

Melatonin in Extrapineal Tissues

Immunohistochemistry has been used to investigate localization of melatonin in several tissues other than the pineal gland. In the retina melatonin has been identified using a variety of antisera (Bubenik et al, 1976c; Vivien Roels et al, 1981). In the retina melatonin is localized in or adjacent to the outer nuclear layer. In the rat pup retina melatonin is already detectable by day two increasing to adult levels by day 20 (Bubenik et al, 1978). Administration of melatonin (by injection or implantation) leads to an increase in retinal melatonin. This increase indicates that the retina is capable of taking up melatonin from the circulation and that it may contain a melatonin binding protein.

Diurnal variation of immunoreactive retinal melatonin levels has been demonstrated (Bubenik et al, 1978; Grota et al, 1982). The presence of melatonin in retina has been confirmed by thin layer

chromatography (Pevet et al, 1980) and by radioimmunoassay (Pang et al, 1977; Reiter et al, 1980).

Synthesis of melatonin has been reported in retina of trout, hamsters and moles (Gern and Ralph, 1979; Pevel et al, 1980, 1981) and the enzymes HIOMT (Cardinali and Rosner, 1971) and NATase (Binkley et al, 1979) have been demonstrated in that tissue.

Melatonin has also been demonstrated in the acinar cells of the Harderian gland by immunohistology and the amount appeared to correlate with the amount of porphyrin in the acini (Bubenik et al, 1976b). Diurnal variation of melatonin has also been shown in the Harderian gland as well as an increase in melatonin following administration (Bubenik et al, 1978). Melatonin synthesis has been reported in both hamsters and mole Harderian glands (Pevet et al, 1980, 1981). HIOMT has been shown in Harderian gland (Cardinali and Rosner, 1972; Pevet et al, 1980) but direct demonstration of NATase has not been achieved to date. The presence of melatonin in Harderian gland has been confirmed by radioimmunoassay (Pang et al, 1977).

Immunoreactive melatonin has also been visualized in the gastrointestinal tract. It is found in the epithelial layer and although it has not been possible to identify the cell type, the localization corresponds to that of the enterochromaffin cells which are known to contain serotonin (Bubenik et al, 1977). No diurnal variation is found in immunoreactive melatonin in colon (Holloway et al, 1980). HIOMT activity has been reported in gut (Quay and Ma, 1976) but the presence of NATase has yet to be studied.

Melatonin has been demonstrated by immunohistochemistry in the suprachiasmatic nucleus of the hypothalamus (Bubenik et al, 1976c) and in the hypothalamus (Grota et al, 1982). The 24 hour pattern of hypothalamic melatonin immunofluorescence appears to be a composite of the retinal and serum rhythms (Grota et al, 1982).

Melatonin in the Circulation

The presence of melatonin in several tissues outside of the pineal gland raises the possibility that these tissues might give rise to circulating melatonin. Indeed studies have reported that pinealectomy fails to eliminate radioimmunoassayable melatonin from the circulation in the rat (Ozaki and Lynch, 1966; Yu et al, 1981) and the sheep (Kennaway et al, 1977).

Recent studies using gas chromatography-mass spectroscopy with negative chemical ion detection (GCMS-NCI) have shown the virtual disappearance of plasma melatonin in the rat (Lewy et al, 1980) and of urinary 6-hydroxymelatonin in the rhesus monkey (Tetsuo et al, 1982) following pinealectomy. Using a radioimmunoassay for serum melatonin which has been crossvalidated with GCMS-NCI (Grota et al, 1981b) we also find that serum melatonin levels in the rat are below the limit of assay sensitivity following pinealectomy (Brown et al, 1983b). It is concluded that under these circumstances the pineal is the major source of circulating melatonin.

Target Sites for Melatonin

Several reports have implicated the pineal hormone, melatonin, in the regulation of neuroendocrine function and behaviour (Datta and King, 1978; Cardinali, 1981; Brown and Niles, 1983); however the sites and mechanisms involved await clarification. In attempts to identify the potential target sites for melatonin, various investigators have carried out binding studies with the tritiated hormone.

In 1978, specific high affinity binding sites for [^{3}N] melatonin were reported to be present in the 105,000 g supernatant fractions from human, rat and hamster ovaries (Cohen et al, 1978). Specific binding was maximal after 3 hours of incubation, however, the temperature used was not mentioned. Binding was heat labile with a complete loss observed after incubation at 70^{o} for 10 min.

Analysis of binding data for hamster cytosol indicated two classes of binding sites (Table 1). Melatonin and 6-fluoromelatonin readily inhibited binding while other indoles showed lower affinity: 5-methoxyindole > 5-hydroxytryptamine > 5-methoxytryptamine. Binding was not affected by tryptophan, 4,6-difluoromelatonin, histamine, dopamine or epinephrine. On the basis of these preliminary findings, the authors suggested that binding requires the indole nucleus and that less affinity is exhibited by indoles lacking the N-acetyl group.

While Cohen and coworkers (1978) did not detect specific binding of ^{3}N-melatonin in whole brain cytosol, Niles and coworkers (1979b) observed binding in cytosol fractions prepared from various rat brain regions. This binding was saturable at 40-50 nM ^{3}N-melatonin and was maximal after incubation for 2 1/2 hours at 37^{o}C (unpublished observations). One class of high affinity sites was detected in the hypothalamus, hippocampus and striatum (Table 1). In the midbrain binding was of low affinity (Kd = 302 nM) and high capacity (B_{max} = 1370 fmol/mg protein).

In contrast to the foregoing, Cardinali and coworkers reported the presence of high affinity sites for ^{3}H-melatonin in crude membrane preparations from bovine, rat and hamster brains (Cardinali et al, 1979; Vacas and Cardinali, 1979). In bovine membranes, binding was highest in the medial basal hypothalamus and structural specificity studies indicated that the 5-methoxy substituent was necessary for high affinity binding (Cardinali et al, 1979). This structural requirement has also been observed in more recent studies which indicated similar Kd values but significantly higher binding site concentrations in rat brain membranes subjected to multiple washing (Niles, 1983).

More work remains to be done in order to fully characterize the binding sites for ^{3}H-melatonin and to determine whether or not these sites are receptors involved in producing the biologic effects of melatonin. Preliminary evidence that the concentration of binding sites in rat and hamster brains is higher late in the photoperiod (Vacas and Cardinali, 1979) when melatonin injections are most effective in influencing neuroendocrine function (Reiter et al, 1976) and that melatonin alters adenylate cyclase activity in rat brain membranes (Niles, 1983), offers support for the physiologic relevance of melatonin binding sites.

TABLE 1

Characteristics of [^{3}H]-melatonin binding in cystosol and membrane fractions from various species and tissue

Species and Tissue	Kd_1 nM	$Bmax_1$ fmol/mg/prot	Kd_2 nM	$Bmax_2$ fmol/mg/mt	Reference
Hamster ovarian cytosol	6.3	52	550	419	21
Rat hypothalamic cytosol	8.6	78	-	-	46
Rat hippocampal cytosol	11.3	166	-	-	46
Rat striatal cytosol	28	61	-	-	46
Bovine hypothalamic membranes	12	14	-	-	19
Rat brain membranes	77	94	-	-	76
Hamster brain membranes	53	50	-	-	76
Rat brain membranes	15	1200	-	-	47

N-Acetylserotonin in the CNS Tissues Other Than the Pineal Gland

In the last decade immunohistochemical procedures have been used extensively to localize and trace the neural pathways of neurotransmitters. Until a few years ago the fluorescence condensation methods were the only available techniques for the visualization of monoaminergic amines in tissues. The development and utilization of antisera to the indoleamine: serotonin, melatonin and N-acetylserotonin (Grota and Brown, 1974; Steinbusch, 1978, 1981; Brown et al, 1983a; Pulido et al, 1983) marked a step forward in indoleamine research. The application of these antisera to immunohistochemistry and radioimmunoassay (RIA) have made possible their measurement (Pang et al, 1983a,b; Pulido et al, 1981, 1983a). N-acetylserotonin (NAS) is an indole initially recognized as a product of the pineal gland. However, a wide distribution through the central nervous system has been demonstrated using immunohistochemistry (Bubenik et al, 1974; Porietis et al, 1978; Pulido et al, 1981, 1983a; Brown et al, 1983a,b) and radioimmunoassay (Pang et al, 1977, 1981). The specificity of the NAS antisera and the validity of the immunohistochemical procedures and of the radioimmunoassay (RIA) have been investigated extensively (Pulido et al, 1983; Brown et al, 1983a,b; Pang et al, 1981).

Detailed comparison between the distribution of INAS containing structures and those containing serotonin showed major differences (Pulido et al, 1983, Brown et al, 1983a). Furthermore no melatonin has been visualized within those brain areas where INAS has been localized (Brown et al, 1983a).

INAS has been demonstrated in hippocampus by immunohistochemistry (Porietis et al, 1978; Brown et al, 1983a, Psarakis et al, 1982) and confirmed by gas chromatography-mass spectometry (GCMS) (Brown et al, 1981). Serotonin localization has also been widely reported in the hippocampus (Steinbusch, 1981). However, the distribution of INAS is substantially different from that of 5-HT. INAS, but not 5-HT, has been localized within cell bodies of the pyramidal cell layers of CA_1 and CA_3 as well as in the granular cell layer of the CA_4 dentate region (Brown et al, 1983a). The presence of INAS in the granular cell layer of the dentate gyrus has been shown to be age-dependent. It appears as early as 20 days post-conception and reaches adult levels by 38 days post-conception (Psarakis et al, 1982).

INAS is present in significant amounts in the cerebellum where it is localized in the granular layer of the cortex, Purkinje cells and cerebellar nuclei (Pulido et al, 1983a). A similar distribution has been observed in several rat species and in human (Pulido et al, 1983a; Brown et al, 1983a). No melatonin containing structures have been localized in cerebellum (Brown et al, 1983a). Serotonin has been reported in very low amounts in the cerebellum and only in nerve terminals (Steinbush, 1981). The presence of INAS in the granular layer of the rat cerebellum has shown to be regulated by a B-adrenergic mechanism similar to that of the pineal gland (Pulido et al, 1983b). Preliminary investigations using young pigmented rats maintained on 12L:12D cycle have shown that INAS in cerebellum maintains constant levels in light and dark (Pulido and Joshi, 1984).

INAS has a wide distribution throughout the brain stem. A detailed mapping of this distribution has been published (Pulido et al, 1983a). Among the most conspicuous structures containing INAS are the locus coeruleus, cochlear nuclei, vestibular nuclei, nuclei pontis and the reticular substance (Pulido et al, 1983a; Brown et al, 1983a).

In addition to the above mentioned brain regions, INAS has recently been demonstrated in the photoreceptor cell and granular layers of the retina of pigmented rats (Pulido and Clifford, 1984).

N-acetylserotonin in the Circulation

It is well recognized that N-acetylserotonin (NAS) is produced in the pineal gland by the action of N-acetyltransferase (NATase) on serotonin (Weissbach et al 1960). Its presence in the pineal gland has also been established by fluorometry (Miller and Maikel 1970), gas-chromatograph-mass spectrometry (Cattabeni et al 1972; Kowslow and Green 1973) and radio enzymatic assay (Klein and Weller 1972; Brownstein et al 1973). Initial interest in this indole has mainly focussed on its role as the substrate for the synthesis of melatonin, the established pineal hormone, and investigations into the physiological role of NAS outside the pineal gland have been few. This was probably due to lack of a simple methodology for its quantification and perhaps also due to its being overshadowed by the popularity of MT.

The presence of NAS in the Harderian gland and cerebellum was demonstrated by an immunohistochemical technique in 1976 (Bubenik et al 1976). However it was not until 1977 when the first radioimmunoassay for NAS became available (Pang et al 1977). In this assay, a bi-specific antibody for MT plus NAS and a monospecific antibody for MT were used. NAS levels in tissues were estimated by a subtraction method. Using this method it was demonstrated that NAS has a wide distribution outside the pineal as it was found in the retina, Harderian gland brain and serum (Pang et al 1977). Subsequently a diurnal rhythm of NAS in rat serum was also demonstrated with high levels from mid-dark to the early period (Pang et al 1980). This finding lead to the initial suggestions that the pineal may be a major source of the circulating NAS, and that NAS may be co-secreted together with MT. This suggestion also correlated with earlier reports that in vitro, NAS is readily secreted into incubation media by rat pineal (Klein et al 1970; Backstrom and Wetterberg 1973) or rat pinealocytes (Ruse et al 1976).

Recently a more specific radioimmunoassay for NAS was developed (Pang et al 1981a). Using this improved methodology, a more detailed study of the regulation of circulating NAS by environmental lighting was performed. Rats were subjected to long (14:10 LD) and short (2:22 LD) light:dark cycles for 6 weeks and the rhythms of serum NAS and MT were determined. It was found that under the short photoperiod the serum NAS and MT rhythms can be dissociated in the rat such that while the peak level of MT remained at the end of the dark period, the peak level of NAS occurred at the middle of the dark period (Grota et al 1981a). This dissociation indicated that either the pineal NATase and HIOMT activities peak at different times under the short photoperiod, or other regulatory factor(s) outside the pineal may be involved in the regulation of serum NAS. However, subsequent experiments show that under both long or short

photoperiods, serum MT and pineal NATase always remain in phase with each other (Ho et al 1984). Thus the earlier reported dissociation of serum NAS and serum MT cannot be due to the dissociation of pineal NATase and serum MT. To further the study of the regulation of serum NAS rhythm, the possibility that the time of feeding can act as a synchronizer for the rhythm, as it does to other biological rhythm, was investigated (Ho et al 1983). Preliminary data indicted that under the usual laboratory lighting conditions (LD 14:10), restriction of food availability to a 3 hour period in the morning or in the evening, can synchronize the serum NAS rhythm such that its peak levels appear at the time of food presentation. This schedule of feeding has no effect on the serum MT rhythm, which remained synchronized with the environmental light:dark cycle. Hence these preliminary findings suggest that periodic access to food is a more potent entrainer than environmental light:dark cycles for the serum NAS rhythm, and strongly suggests that the pineal is not the major source of circulating NAS. This finding therefore correlates well with the previous work which shows that serum NAS is not abolished by pinealectomy (Yu et al 1981).

The major source(s) of circulating NAS remains to be elucidated at the present time. One of the likely sources is the retina. The activities of retinal NATase (Binkley et al 1979) and levels of N-acetylserotonin (Pang et al 1981) have been shown to exhibit a diurnal rhythm. Recently it has been demonstrated that NAS can also be released from the retina of the guinea pig in vitro (Yu et al 1982). Other possible sources of circulating NAS are the Harderian gland and the brain where the presence of NAS has been demonstrated by immunohistochemistry (Bubenik et al 1976a, 1976b; Pulido et al 1983a). Certainly other organs which possess the enzyme N-acetyltransferase can also be a possible source of circulating NAS. These include the liver, adrenal gland, submaxillary gland, kidney, heart, pituitary, thyroid and spleen (Ellison et al 1972). It is possible that circulating NAS can originate from several sources. It is interesting to note that a recent publication reports that exogenous MT, when given to rats, can be metabolised to NAS as determined by GC-MS (Leone and Silman 1984). However in that study, pharmacological doses of MT were used and whether NAS can be a significant metabolite of MT under normal physiological conditions remains to be determined.

Although detailed studies on the importance of NAS in the body economy have been few, there are enough data accumulated to suggest that it may have a significant physiological role. Studies by Vaughan et al (1972) showed that NAS inhibited compensatory ovarian hypertrophy in female mice. Immunization of male rats against NAS-M-BSA suppressed corticosterone (Niles et al 1977a), prolactin (Niles et al 1977b) and testosterone (Niles et al 1979a). Sleep changes induced by 6-F-tryptophan were prevented by administration of NAS but not by 5-OH-tryptophan (Sugden and Fletcher 1981). Earlier study has shown that NAS treatment inhibited ^{131}I uptake into the thyroid gland (De Prospo et al 1969) and recently injection of small doses of NAS was demonstrated to be able to significantly depress serum TSH levels (Tang and Pang 1983). The findings that circulating NAS does not originate mainly from the pineal gland, that its rhythm in the serum can be synchronized with the time of food presentation, together with the demonstrated physiological effects of this indole, strongly suggests that NAS may have an important function of its own right and hence warrant

more detailed studies. Perhaps more fruitful results can be obtained if future studies can be dissociated from the premise that circulating NAS is closely related to the pineal.

Target Sites for NAS

As discussed earlier, the melatonin precursor NAS, and its synthesizing enzyme NAT, are present in the rat CNS which suggests that this putative neurohormone may be synthesized centrally. In order to determine the possible CNS target sites for NAS, we have examined the binding characteristics of [^{3}H] NAS in mammalian brain fractions. High affinity binding was detected in synaptosomal membranes from human, calf and rat brain.

One class of binding sites, with a dissociation constant (Kd) of 3-7 nM and a concentration of 250-400 fmol/mg protein, was detected in fresh brain membranes from male and female rats. A similar high affinity class of binding sites together with a second class of low affinity and high capacity sites were observed in frozen membranes (Niles et al, 1982).

A comparison of the regional binding of [^{3}H] NAS and [^{3}H]5-HT indicated that [^{3}H]5-HT binding was about 3-10 fold greater than that of [^{3}H] NAS. Both radioligands exhibited highest binding in the striatum and frontal cortex, however there were differences in the relative binding levels observed in other brain regions. With striatal binding regarded as 100%, [^{3}H] NAS binding was 66%, 46% and 37% in the cerebellum, pons-medulla and midbrain respectively, while corresponding levels for [^{3}H]5-HT were 46%, 25% and 25%. Conversely, [^{3}H]5-HT binding was higher in the hypothalamus (85%) and hippocampus (71%) as compared with 67% and 41% for [^{3}H] NAS. These findings indicate relatively more binding sites for [^{3}H] NAS are present in the cerebellum and brainstem (Niles et al, 1983).

Displacement studies, carried out in fresh cortical membranes, indicated that NAS and 5-HT were the most potent inhibitors of [^{3}H] NAS binding (Table 2). The Hill coefficients for NAS and 5-HT were approximately equal to unity, suggesting a single class of binding sites. Analogs with a methoxy group in place of the 5-hydroxyl showed a marked decrease in affinity and in the absence of a C-5 substituent there was no binding.

The 5-HT agonist, quipazine, and various anti-serotoninergic drugs including the 5-HT$_2$ antagonists, spiperone and ketanserine, displayed intermediate affinity for [^{3}H] NAS binding sites (Table 2). Taken together, the foregoing suggests that the 5-hydroxyl group is necessary for high affinity binding of [^{3}H] NAS which occurs at both 5-HT$_1$ and 5-HT$_2$ sites in the rat frontal cortex.

Nonetheless, differences between the affinities of various 5-HT antagonists against [^{3}H]5-HT (Nelson et al, 1978; Peroutka and Snyder, 1979) and [^{3}H] NAS (Niles et al, 1983) and in the relative binding of these radioligands in the brainstem and cerebellum, suggest that non-serotoninergic binding sites for [^{3}H] NAS are also present in the CNS.

TABLE 2

Displacement of specifically bound [^{3}H] NAS
from rat cortical membranes

Drug	$IC_{50(nM)}$	nH
N-acetylserotonin	13.5 + 2.9	1.1 + 0.1
Serotonin	2.2 + 1.9	1.0 + 0.1
Quipazine	190	0.49
5-methoxytryptamine	430	0.82
Melatonin	> 10,000	0.39
N-acetyltryptamine	ND	
Methysergide	80	0.86
Ketanserine	118	0.98
Spiperone	158	0.64
Cyproheptadine	5,348	0.64

Fresh synaptic membranes were incubated with 2.5 nH of [^{3}H] NAS at least five concentrations of each drug. Nill coefficients (nH) are equal to the slopes of logit-log plots. Means + S.E.M. of 2-5 experiments are presented.

ND = no displacement

SUMMARY AND CONCLUSION

The pineal hormone melatonin is present in a variety of extrapineal tissues including the retina, Harderian gland and gastrointestinal tract. For the retina, there is excellent evidence of local synthesis while for the other tissues evidence is less complete.

Following pinealectomy, melatonin virtually disappears from the circulation and the metabolite 6-hydroxymelatonin is almost completely absent from urine.

Binding sites for melatonin have been described: cytosol binding is evident in ovarian and brain tissue and membrane binding is present in brain tissue. Circadian variation of melatonin binding in brain has been reported as well as alteration of adenylate cyclase activity.

Taken together these findings suggest that melatonin, like serotonin, has a variety of origins and actions. Melatonin produced in the pineal is the major source of circulating melatonin and major sites of action may be the brain and ovaries; melatonin in other tissues usually contributes little to circulating melatonin and instead may have local actions as a modulator.

N-acetylserotonin is also found in a variety of tissues other than the pineal gland. It is present in the retina and Harderian gland and it is present in a variety of brain regions, notably including the granular layer of cerebellum and a variety of motor and reticular nuclei in the brain stem.

N-acetylserotonin is present in the circulation in concentrations 20 to 50 times higher than those of melatonin, is regulated differently from melatonin under various photoperiodic and feedings conditions and is relatively unaltered following pinealectomy. Thus, circulating NAS for the most part originates in tissues other than the pineal. Although the site of origin is as yet not known, several organs contain NATase, the enzyme required for NAS synthesis.

High affinity membrane binding of NAS has been shown in various brain regions, and there is evidence that NAS binds to serotonergic sites. However, pharmacologic studies suggest that non-serotonergic binding sites for NAS are also present.

From a consideration of these findings it may be concluded that NAS, like serotonin and melatonin, is present in a variety of tissues and may have several functions. In the pineal gland and presumably in other tissues in which melatonin is synthesized, it is a precursor for melatonin; in other tissues such as brain it may have functions independent of that of melatonin precursor.

REFERENCES

1. Axelrod J and Weissbach H (1961) Purification and properties of hydroxyindole-O-methyltransferase. J. Biol. Chem. 236: 211-213.

2. Backstrom M and Wetterberg L (1973) Increased N-acetylserotonin and melatonin formation induced by D-amphetamine in rat pineal culture via an -adrenergic receptor mechanism. Acta physiol. scand. 87:3-120.

3. Binkley S, Hryshchyshyn M and Reilly K (1979) N-acetyltransferase activity responds to environmental lighting in the eye as well as in the pineal gland. Nature Lond. 28:9.

4. Binkley S, Reilly K, Hernandez T (1980) N-acetyltransferase in the chick retina. J. Comp. Physiol. 140:1-183.

5. Brown GM, Grota LJ, Pulido O, Burns TG, Niles L and Snieckus V (1983b) Application of immunologic techniques to the study of pineal indolealkylamines. Pineal Res. Rev. 1:7-247.

6. Brown GM and Niles LP (1983) Studies on melatonin and other pineal factors. In: Besser GM and Martini L (eds). Clinical Neuroendocrinology, Vol 11, pp. 205-264, Academic Press, New York.

7. Brown GM, Porietis AV and Narasimhachari N (1981) Identification and quantification of N-acetylserotonin in rat brain regions by GCMS. Society of Neuroscience Abstracts, 7: 580.

8. Brown GM, Pulido O, Niles LP, Psarakis S, Porietis A, Bubenik GA and Grota LJ (1983a) Differential localization of melatonin and N-acetylserotonin in brain. In: The Pineal Gland and its Endocrine Role. Axelrod J, Fraschini F and Velo GP (Eds.), Plenum Press, New York, 1 pp. 257-276.

9. Brownstein MJ (1975) Minireview. The Pineal Gland. Life Sciences 16:1363-1374.

10. Brownstein M, Saavedra JA and Axelrod J (1973) Control of pineal N-acetylserotonin by a beta adrenergic receptor. Mol. Pharmacology 9:605-611.

11. Bubenik GA, Brown GM, Uhlir I and Grota LJ (1974) Immunohistological localization of N-acetylindolealkylamines in pineal gland, retina and cerebellum. Brain Res. 81:233-242.

12. Bubenik GA, Brown GM and Grota LJ (1976a) Immunohistological investigations of N-acetylserotonin (NAS) in the cerebellum after parachlorophenylalanine (PCPA) treatment. Experientia 32: 579-580.

13. Bubenik GA, Brown GM and Grota LJ (1976b) Immunohistochemical localization of melatonin in the rat Harderian gland. J. Histochem. Cytochem. 24:1173-1177.

14. Bubenik GA, Brown GM and Grota LJ (1976c) Differential localization of N-acetylated indolealkylamines in CNS and the Harderian gland using immunohistology. Brain Res. 118:417-427.

15. Bubenik GA, Brown GM and Grota LJ (1977) Immunohistological localization of melatonin in the rat digestive system. Experientia, 33:662-663.

16. Bubenik GA, Purdill RA, Brown GM and Grota LJ (1978) Melatonin in the retina and the Harderian gland. Ontogeny, diurnal variation and melatonin treatment. Exp. Eye Res. 27:323-333.

17. Cardinali DP (1981) Melatonin. A mammalian pineal hormone. Endocrine Rev. 2(3):327-346.

18. Cardinali DP and Rosner JM (1971) Retinal localization of hydroxyindole-O-methyltransferase (HIOMT) in the rat. Endocrinol. 89:301-303.

19. Cardinali DP, Vacas MI and Boyer EE (1979) Specific binding of melatonin in bovine brain. Endocrinology 105:437-441.

20. Cattabeni F, Koslow SH and Costa E (1972) Gas chromatographic-mass spectrometric assay of four indole alkylamines of rat pineal. Science 178:166-168.

21. Cohen M, Roselle D, Chabner B, Schmidt TJ and Lippman M (1978) Evidence for a cytoplasmic melatonin receptor. Nature 274: 894-895.

22. Datta PC and King MG (1978) Melatonin: effects on brain and behaviour. Neurosci. Behav. Rev. 4:451-458.

23. Ellison N, Weller JL and Klein DC (1972) Development of a circadian rhythm in the activity of pineal serotonin N-acetyl-transferase. J. Neurochem. 19:1335-1341.

24. Erspamer V (1966) 5-hydroxytryptamine and related indolealkylamines. Handbook of Experimental Pharmacology, Vol. XIX, Springer, Berlin, Heidelberg, New York 928 pp.

25. Gaddum JH (1936) Gefaesseriweiternde Staffe der Gwebe, Thieme-Verlage, Leipig.

26. Gern WA, Ralph CL (1979) Melatonin synthesis by retina. Science 204:183-184.

27. Grota LJ and Brown GM (1974) Antibodies to indolealkylamines: serotonin and melatonin. Can. J. Biochem. 52:196-202.

28. Grota LJ, Brown GM and Pang SF (1981a) Dissociation of N-acetylserotonin and melatonin levels in serum of rats. 11th Annual Meeting of the Society of Neurosciences, p. 717 (Abstract).

29. Grota LJ, Snieckus V, deSilva SO, Tsui HW, Holloway WR, Lewy AJ and Brown GM (1981b) Radioimmunoassay of melatonin in rat serum. Prog. Neuro-psychopharmacol. 5:523-526.

30. Grota LJ, Holloway WR and Brown GM (1982) 24 Hours rhythm of hypothalamic melatonin immunofluorescence correlates with serum and retinal melatonin rhythms. Neuroendocrinol. 34:363-368.

31. Ho AK, Burns TG, Grota LJ and Brown GM (1983) Differential effects of scheduled feeding on melatonin, N-acetylserotonin and corticosterone rhythms in rat serum. 13th Annual Meeting of the Society of Neurosciences, p. 313.12.

32. Ho AK, Grota LJ and Brown GM. (1984) Relationship between pineal N-acetyltransferase activity, pineal melatonin and serum melatonin in rats under different lighting conditions. Accepted for publication, Neuroendocrinology.

33. Holloway WR, Grota LJ and Brown GM (1980) Determination of immunoreactive melatonin in the colon of the rat by immuno-cytochemistry. J. Histochem. Cytochem. 28:255-262.

34. Kennoway DJ, Drith RG, Phillipou G, Matthews CD and Seamark RF (1977) A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography - mass spectroscopy. Endocrinology 101:119-127.

35. Klein DC, Berg GR and Weller JL (1970) Melatonin synthesis: adenosine 3'5'-monophosphate and norepinephrine stimulate N-acetyltransferase. Science 168:979-980.

36. Klein DC and Weller JL. Pineal N-acetylserotonin. Fed. Proc. Fed. Am. Soc. exp. Biol. 31:222.

37. Koslow SH and Green AR (1973) Analysis of pineal and brain indole alkylamines by gas chromatography-mass spectrometry. Adv. biochem. Psychopharmac. 7:33-43.

38. Leone RM and Silman RE (1984) Melatonin can be differentially metabolised in the rat to produce N-acetylserotonin in addition to 6-hydroxymelatonin. Endocrinology 114:1825-1832.

39. Lerner AB, Case Jd, Takahashi Y, Lee TH and Mari W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J. Amer. Chem. Soc. 80: 2587.

40. Lewy AJ, Tetsuo M, Markey SP, Goodwin FK and Kopin I (1980) Pinealectomy abolishes plasma melatonin in the rat. J. Clin. Endocrinol. Metab. 50:204-205.

41. Miller FP and Maickel RP (1970) Fluorometric determination of indole derivatives. Life Sci. 9:747-752.

42. Nelson DL, Herbet A, Bourgoin S, Glowinski J and Hamon M (1978) Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat. Mol. Pharmacol. 14:983-995.

43. Niles LP, Brown GM and Grota LJ (1977a) Endocrine effect of the pineal gland and neutralization of circulating melatonin and N-acetylserotonin. Can. J. Physiol. Pharmacol. 55:537-544.

44. Niles LP, Brown GM and Grota LJ (1977b) Effects of neutralization of circulating melatonin and N-acetylserotonin or plasma prolactin levels. Neuroendocrinology 23:14-22.

45. Niles LP, Brown GM and Grota LJ (1979a) Role of the pineal gland in diurnal endocrine secretion and rhythm regulation. Neuroendocrinology 29:14-21.

46. Niles LP, Wong Y-W, Mishra RK and Brown GM (1979b) Melatonin receptors in brain. Eur. J. Pharmacol. 55:219-220.

47. Niles LP (1983) Melatonin binding in brain membranes and effects on adenylate cyclase. Neurosci. Abstracts.

48. Niles LP, Brown GM and Mishra RK (1982) A serotonergic component of [^{3}H]N-acetylserotonin binding in mammalian brain. Prog. Neuro-Psychopharmacol. Biol. 6:421-424.

49. Niles LP, Brown GM and Mishra RK (1983) Characteristics of high affinity binding of [^{3}H]N-acetylserotonin in rat brain. Neuropharmacol. 22(11):1311-1314.

50. Osborne NN (1982) Assay, destribution and function of serotonin in nervous tissues. In: Biology of Serotonergic Transmission, NN Osborne (ed.), pp. 7-27, John Wiley and Sons, New York.

51. Ozaki Y and Lynch HJ (1976) Presence of melatonin in plasma and urine of pinealectomized rats. Endocrinology 99:641-644.

52. Page IH (1954) Serotonin (5-hydroxytryptanine). Physiol. Rev. 34:563-568.

53. Pang SF, Brown GM, Grota LJ, Chambers JW and Rodman RL (1977) Determination of N-acetylserotonin and melatonin activities in the pineal gland, retina, Harderian gland, brain and serum of rats and chickens. Neuroendocrinology, 23:1-12.

54. Pang SF, Yip MK, Lui HW, Brown GM and Tsui HW (1980) Diurnal rhythms of immunoreactive N-acetylserotonin and melatonin in the serum of male rats. Endocrinologica 95:571-576.

55. Pang SF, Brown GM, Campbell SL, Snieckus V, DeSilva SO, Young SH and Grota LJ (1981a) A radioimmunoassay of N-acetylserotonin for biological tissue. J. Immunoassay 2:263-276.

56. Pang SF, Yu HS and Tang PL (1981b) A diurnal rhythm of N-acetylserotonin in the retina of rats. Neurosci. Lett. 21:197-200.

57. Peroutka SJ and Snyder SH (1979) Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}]lysergic acid diethylamide and [^{3}H]spiroperiodol. Mol. Pharmacol. 16:687-699.

58. Pevet P, Balemans MGM, Legerstee WC, Vivien Roels B (1980) Circadian rhythmicity of activity of hydroxyindole-0-methyl- transferase (HIOMT) in the formation of melatonin and 5-methyl-tryptophol in the pineal, retina and Harderian gland of the golden hamster. J. Neural Transm. 49:229-245.

59. Pevet P, Balemans MGM, de Reuver GF (1981) The pineal gland of the mole (Talpa europea L.) VII - Activity of hydroxyindole-0-methyltransferase (HIOMT) in the formation of 5-methoxytryptophan, 5-methoxytryptamine, 5-methoxyindole-3-acetic acid, 5-methoxy-tryptophol and melatonin in the eyes and the pineal gland. J. Neurol. Transm. 51:271-282.

60. Porietis AV, Brown GM and Grota LJ (1978) Immunohistochemical localization of N-acetylserotonin in rat hippocampus. Society for Neuroscience Abstracts 4:226.

61. Psarakis S, Pulido O, Brown GM, Grota LJ and Smith GK (1982) Identification and quantification of N-acetylserotonin (NAS) in developing hippocampus of the rat. Prog. Neuropsychopharmacol. and Biol. Psychiat. 6:439-442.

62. Pulido O, Brown GM and Grota LJ (1981) Localization of N-acetylserotonin (NAS) in the rat hindbrain by immunohistology. Prog. Nueropsychopharmacology 5:573-576.

63. Pulido O, Brown GM and Grota LJ (1983a) An immunohistochemical method for the localization of N-acetylserotonin (NAS) in the central nervous system. J. Histochem. Cytochem. 31:1343-1350.

64. Pulido O, Brown GM and Grota LJ (1983b) B-adrenergic regulation of N-acetylserotonin (NAS) synthesis in the rat cerebellum. Life Sciences 33:1081-1089.

65. Pulido O and Joshi M (1984) Effect of light and dark on immunoreactive N-acetylserotonin in the rat cerebellum. (In preparation)

66. Pulido O and Clifford J (1984) Localization of N-acetylserotonin in the rat retina as compared to melatonin. (In preparation)

67. Quay WB and Ma YH (1976) Demonstration of gastrointestinal jydroxyindole-0-methyltransferase. IRCS Med. Sci. 4:563.

68. Reiter RJ, Blask DE, Johnson LY, Rudeen PK, Vaughan MK and Waring PJ (1976) Melatonin inhibition of reproduction in the male hamster: its dependency on time of day of administration and on an intact and sympathetically innervated pineal gland. Neuroendocrinology 22:107-110.

69. Reiter RJ, Richardson BA, Hurlbut EC (1980) Pineal, retina and

Harderian gland melatonin in a diurnal species, the Richardson's ground squirrel (Spermophilus richardsonii). Neurosci. Lett. 22:285-288.

70. Ruse J, Brown GM and Rodman R (1976) In vitro production of N-acetylindoleamines by the pineal gland. 6th Annual Meeting of the Society of Neurosciences, p. 983 (Abstract).

71. Steinbusch HWM (1981) Distribution of serotonin immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6:557.

72. Sugden D and Fletcher A (1981) Changes in the rat sleep-wake cycle produced by DL-6-fluorotryptophan, a competitive inhibitor of tryptophan hydroxylase. Psychopharmacology 74:369-373.

73. Tang F and Pang SF (1983) Effect of N-acetylserotonin on the serum level of thyroid-stimulating hormone in the male rat. Neuroendocrinology 36:268-271.

74. Tetsuo M, Perlow MJ, Mishkin M and Markey SA (1982) Light exposure reduces and pinealectomy virtually stops urinary excretion of 6-hydroxymelatonin by rhesus monkeys. Endocrinology 110:997-1003.

75. Twarog BM and Page IH (1953) Serotonin content of some mammalian tissues and urine and a method for its determination. Am. J. Physiol. 175:157-161.

76. Vacas MI and Cardinali DP (1979) Diurnal changes in melatonin binding sites of hamster and rat brains. Correlation with neuroendocrine responsiveness to melatonin. Neurosci. Lett. 15:259-263.

77. Vaughan MK, Reiter RJ, Vaughan GM, Bigelow L and Altschule MD (1972) Inhibition of compensatory ovarian hypertrophy in the mouse and vole: a comparison of Setschule's pineal extract, pineal indoles, vasopressin and oxytocin. Gen. compar. Endocr. 18: 372-377.

78. Vivien-Roels B, Pevet P, Dubois MP, Arendt J and Brown GM (1981) Immunohistochemical evidence for the presence of melatonin in the pineal gland, the retina and the Harderian gland. Cell Tissue Res. 217:105-115.

79. Weissbach H, Redfield BG and Axelrod J (1960) Biosynthesis of melatonin, enzyme conversion of serotonin to N-acetylserotonin. Biochim. Biophys. Acta 43:352.

80. Yu HS, Pang SF, Tang PL and Brown GM (1981) Persistence of circadian rhythms of melatonin and N-acetylserotonin in the serum of rats after pinealectomy. Neuroendocrinology 32: 262-265.

81. Yu HS, Chow PL, Tang PL and Pang SF (1982) Effect of light and darkness on the in vitro release of N-acetylserotonin and melatonin by retina of guinea pigs. Neuroendocrinology 34: 265-268.

THE PINEAL GLAND OF PRIMATES INCLUDING MAN

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

HUMAN PINEAL GLAND: AN INTRODUCTION

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One of the most dramatic advances in neuroendocrinology of this decade has been the recognition of the importance of the pineal gland. The enormous strides made in this field in experimental research have also strong impact on human studies. Within the span of few years, it has become potentially possible to associate the pineal functions with a number of clinical situations, of which human sexual maturation and affective disorders have been relatively thoroughly investigated.

HISTORICAL:

Investigation of a possible relation between acceleration of pubertal growth and the pineal gland in humans began already about three centuries ago. The first reported observation which may be construed as that of a pineal tumour was apparently in the 17th century by Charles Drelincurtius, which was published in a book in 1717 in Geneva. In the 18th century Giovanni Battista Morgagni, in his well-known compendium (1761) described several patients who had enlarged pineal glands. In 1896, Richard Gutzeit in Königsberg (now Kaliningrad) described in his Dissertation for Doctor of Medicine a 7.75 year old boy with unusually large and precociously mature external genitalia and a teratoma of the pineal gland. During 1898 and 1899 four papers came out relating the pineal gland with precocious puberty. Heubner (1898) and Oestreich and Slawyk (1899) in their papers used the word "Riesenwuchs". The other two cases were presented at the meeting of the Pathological Society of London by Garrod (1899) and Ogle (1899). As early as 1906, Max Askanazy of Geneva described a choriocarcinoma in the pineal region and associated with precocious puberty. He thought that precocious puberty was due to some chemical substances originating in the pineal tumours. During 1909 and 1910 a number of papers, relating the advancement of puberty with the pineal, appeared of which the papers by Lothar von Frankl-Hochwart (1909) in Vienna and Battista Pellizzi (1910) of Pisa are considered to be most important. In 1923 Knud Krabbe of Copenhagen wrote a paper in Endocrinology entitled, "The pineal gland, especially in relation to the problem on its supposed significance in sexual development".

Table I: THE PINEAL AND PUBERTY

FINDINGS:	AUTHORS:
Plasma Melatonin	
1. Daytime MEL declines with puberty in boys	Silman et al. (1979)
2. Daytime MEL level is almost constant during puberty. No sex difference.	Tamarkin et al. (1982) Ehrenkrank et al.(1982) Lenko et al. (1982) Gupta et al. (1983)
3. Episodic release of MEL during daylight in pubertal boys.	Fevre et al. (1978)
4. No change in the 24-hr MEL profile during puberty	Tamarkin et al. (1982) Ehrenkranz et al.(1982)
5. Decline in night-day-difference in MEL level during puberty.	Gupta et al. (1983)
6. Animal Expts.Decline in night-day difference in MEL level during puberty	Reiter et al. (1980) Reiter (1983) Moeller et al. (1983)
CSF Melatonin	
Decline in daytime CSF MEL levels. 8 70 yrs.	Brown et al. (1979)
Urinary Melatonin	
1. MEL excretion increases with pubertal development.	Penny (1982)
2. No change with 6-OH-MEL excretion with pubertal onset except in girls during breast development Stage II. It rises.	Tetsuo et al. (1982)
AGING	
1. Plasma MEL declines with increasing age.	Pelham et al. (1973)
2. Decline in night-level of plasma MEL with aging. 1 92 yrs.	Iguchi et al. (1982)
3. Decline in night-day-rhythm in plasma MEL with aging in adults.	Bartsch et al. (1983)

MODERN RESEARCH TREND IN HUMAN DEVELOPMENT:

In the first presentation of this section, the relationship of human puberty with the pineal gland, defined in terms of serum melatonin value, has been taken up. That this paper was chosen to be the first presentation in the clinical field is a reflection of the significance of studies on puberty in the overall development of pineal physiology and clinical neuroendocrinology.

The data so far obtained in the human studies, however, suggest more confusion than unanimity. Table 1 illustrates the situation.

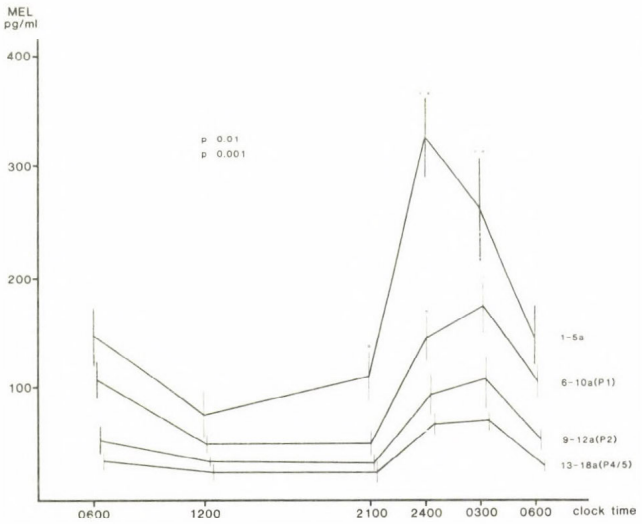

Fig.1: Biorhythm of serum melatonin in children at various stages of pubertal development.

In 1982, we reported at the International Congress of Psychoneuroendocrinology, held in Tübingen, that the amplitude of the net night increment declines with pubertal development. In this study we had 87 children, from whom we collected blood twice, one at day time (12 noon) and the other at midnight (Gupta et al.1983).These data clearly showed that there was a significant ($p < 0.001$) decline in net increment from pubertal stage PI to PII. Obviously,this study had drawbacks, as taking only two time points we must have missed the exact peak in

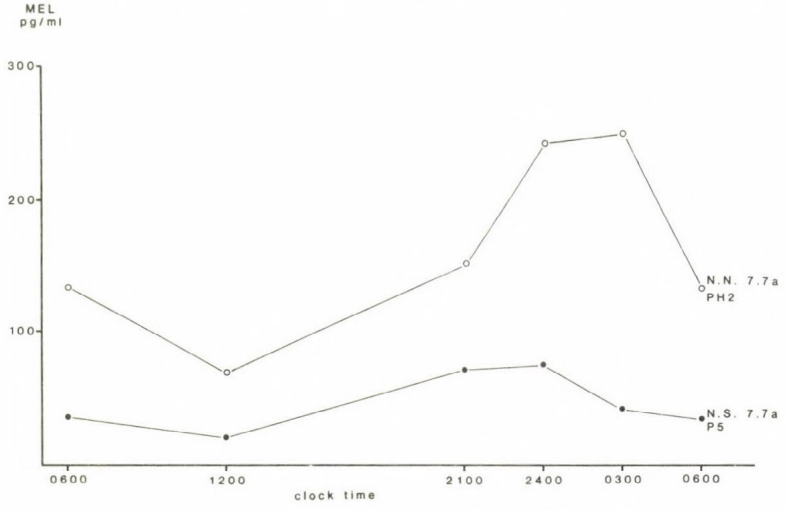

Fig.2: Biorhythm of serum melatonin in twin sisters with different stages of pubertal development (Attanasio et al.1983).

the biorhythm of melatonin. In spite of this, the report suggested a very strong case for an interdependent relation between the pineal gland and pubertal development which was later supported by a couple of reports published (Lissoni et al.1983; Waldhauser et al. 1983).

Recently, we have extended our investigations further (Attanasio et al.in press) where circadian rhythm of serum melatonin was examined under standardised protocol in 38 children grouped in various pubertal developmental stages (Fig.1). This shows clearly that the night increment declines steadily from the level seen in the pre-school children to that in the sexually more mature children. However, in this case the more surprising finding was that the amplitude of the night peak also declines from the pre-school children's level to pubertal stage I level. This suggests that there is a relation between the pineal gland and not pubertal development as such but rather the whole developmental process in children.

One bit of evidence might be drawn from a pathological example. Figure 2 illustrates the biorhythm of melatonin in 2 girls who are twins (Attanasio et al. 1983). They are 7.7 yrs old. One sister (N.S.) started menstruating at age 6.5 years and her pubertal rating was P V. The other sister (N.N.) had mild adrenarche and her pubertal rating was P II. When the biorhythms were compared, the PII girl showed substantial increment in melatonin night level, while the other sister, having advanced sexual maturation, had comparable melatonin rhythm seen in normal P V girls. This suggests again that with sexual development and maturation, even though untimely, the pineal gland is orchestrated together.

AFFECTIVE DISORDERS :

One of the major adaptive mechanisms developed during the course of evolution in response to external environmental changes is the temporal organization of behaviour and of physiological processes. In the absence of synchronizing influence, the endogenous rhythm is disorganized which differs from the pattern imposed upon it by the Zeitgeber. Professor Wetterberg's paper is a significant contribution to the understanding the role of the pineal gland in the genesis of distorted rhythms in mental disorder. The results from his laboratory (Wetterberg,1979) already suggested dysfunction of the pituitary-adrenocortical axis and involvement of the pineal gland in depression. There is also evidence that in bipolar affective disorders, the manic patients had considerably high melatonin levels throughout. It is possible that the increased adrenargic activity in the manic patients probably stimulates the pineal gland as well as the presence of ultradian rhythms may well be involved with the altered receptor regulation in such subjects.

REFERENCES:

Askanazy,M.: Teratom und Chorionepithelium der Zirbel. Verh. Dtsch.Ges.Pathol. 10, 58-76,1906.

Attanasio,A.,Borrelli,P.,Marini,R.,Cambiaso,P.,Cappa,M. and Gupta,D.: Serum melatonin in children with early and delayed puberty. Neuroendocrinol.Lett. 5, 387-392, 1983.

Attanasio,A.,Müller,F.,Borrelli,P. and Gupta,D.: Circadian rhythms in serum melatonin from infancy to adolescence. J.Clin.Endocrinol.Metab. (in press).

Bartsch,C.,Bartsch,H.,Fluchter,S.H.,Harzmann,R.,Attanasio,A., Bichler,K.H. and Gupta,D.: Circadian rhythms of serum melatonin, prolactin, and growth hormone in patients with benign and malignant tumours of the prostate and in non-tumour controls. Neuroendocrinol.Lett. 5, 377-386, 1983.

Brown,G.M., Young,S.N.,Gauthier,S.,Tsui,H.,and Grota,L.J.: Melatonin in human cerebrospinal fluid in daytime: its origin and variation with age.Life Sci. 25,929-936,1979.

Drelincurtius,C.: cited Manget,J.J.: Theatrum,Anatomicum, Cramer and Perachon,Geneva,1717, p.309.

Ehrenkranz,J.R.L.,Tamarkin,L.,Comite,F.,Johnsonbaugh,R.E., Bybee,D.E.,Loriaux,D.L. and Cutler,G.B.: Daily rhythm of plasma melatonin in normal and precocious puberty. J.Clin. Endocrinol.Metab. 55,307-310, 1982.

Fevre,M.,Segel,T.,Marks,J.M. and Boyar,R.M.: LH and melatonin secretion patterns in pubertal boys.J.Clin. Endocrinol.Metab. 47,1383-85,1978.

v.Frankl-Hochwart,L.: Über Diagnose der Zirbeldrüsentumoren. Dtsch.Z.Nervenheilk. 37, 455-465, 1909.

Garrod,A.E. : Pineal cyst.Trans.Pathol.Soc.Lond.50,14-15,1899.

Gupta,D.,Riedel,L.,Frick,H.J.,Attanasio,A. and Ranke,M.B.: Circulating melatonin in children: In relation to puberty, endocrine disorders, functional tests and racial origin. Neuroendocrinol.Lett. 5,63-78, 1983.

Gutzeit,R.: Ein Teratom der Zirbeldrüse. Inaugural Dissertation der medizinischen Facultät zu Königsberg i.Pr.zur Erlangung der Doctorwürde. Erlatis,Königsberg, 1896.

Heubner,W.: Tumor der Glandula pinealis.Dtsch.Med.Wochenschr. 24 (Beilage 29) 214-215, 1898.

Iguchi,M.,Kato,K.I. and Ibayashi,H.: Age dependent reduction in serum melatonin concentrations in healthy human subjects. J.Clin.Endocr.Metab. 55, 27-29, 1982.

Krabbe,K.H.: The pineal gland especially in relation to the problem on its supposed significance in sexual development. Endocrinology, 7, 379-414, 1923.

Lenko,H.L.,Lang,U.,Aubert,M.L.,Paunier,L. and Sizonenko,P.C. Hormonal changes in puberty: VIII. Lack of variation of daytime plasma melatonin. J.Clin.Endocrinol.Metab.54,1056-58,1982.

Lissoni,P.,Resentini,M.,Mavri,R.,Morabito,F.,Djemal,S.and Fraschini,F.:A study of the melatonin circadian rhythm in normal subjects and cases of delayed puberty. J.Endocrinol.Invest. 6,5,1983.

Moeller,H.,Koz,A.,Rödl,W.,Frick,H.J. and Gupta,D.: Role of the pineal gland in the regulation of prostatic androgen receptors in pubertal and mature rats. Res.Exp.Med. 183, 157-165,1983.

Morgagni,J.B.: The seats and causes of diseases investigated by anatomy.Hafner,New York, 1960 (First edition in 1761,Venice)

Oestereich,R. and Slawyk,V.: Riesenwuchs und Zirbeldrüsen-Geschwulst. Virchows Arch. 157, 457-484, 1899.

Ogle,C.:1.Sarcoma of the pineal body, with diffuse melanotic sarcoma of the surface of cerebrum. 2. Tumor of the pineal body in a boy.Trans.Pathol.Soc.Lond.50,4-12, 1899.

Pelham,R.W.,Vaughan,G.M.,Sandock,K.L. and Vaughan,M.K.:Twenty-four-hour cycle of a melatonin-like substance in the plasma of human males. J.Clin.Endocrinol.Metab. 37, 341-344, 1973.

Pellizzi,G.B.:La sindrome epifisaria "macrogenitosomia precoce". Riv.Ital.Neuropatol.Psichiat.Eletroter. 3,193-207,250-272,1910.

Penny,R.: Melatonin excretion in normal males and females: increase during puberty.Metabolism,31, 816-823,1982.

Reiter,R.J.:Changes in mammalian pineal indolamine metabolism during aging. Neuroendocrinol.Lett. 5, 149, 1983.

Reiter,R.J.,Richardson,B.A.,Johnson,L.Y.,Ferguson,B.N. and Dinh,D.T.: Pineal melatonin rhythm:reduction in aging Syrian hamsters. Science, 210, 1372-73, 1980.

Silman,R.E.,Leone,P.M.,Hooper,R.J.L. and Preece,M.A.:Melatonin, the pineal gland and human puberty. Nature, 282, 301-303,1979.

Tamarkin,L.,Abastillas,P.,Chen,H.,McNemar,A. and Sidbury,J.B.: The daily profile of plasma melatonin in obese and Prader-Willi Syndrome children. J.Clin.Endocrinol.Metab. 55,491-495,1982.

Tetsuo,M.,Poth,M. and Markey,S.P.: Melatonin metabolite excretion during childhood and puberty. J.Clin.Endocrinol.Metab. 55, 311-313, 1982.

Waldhauser,F.,Frisch,H.,Weiszenbacher,G.and Wurtman,R.:Serum melatonin and serum LH concentrations in children,adolescents and young adults. Ped.Res.18, 104, 1984.

Wetterberg,L.: Clinical importance of melatonin. Progr. Brain Res. 52, 539-547, 1979.

The Pineal Gland
Current State of Pineal Research
B. Mess, Cs. Rúzsás, L. Tima and P. Pévet (eds)

MELATONIN AND PUBERTY: EXPERIMENTAL AND CLINICAL APPROACHES

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INTRODUCTION

Studies of the physiology of the pineal gland in different vertebrate species have confirmed that this small organ located in the brain is an active neuroendocrine gland which, among other functions, modulates reproductive biology. In animals with a seasonal cycle of reproduction, the pineal gland is responsible for transducing environmental information such as changes in the daylight period into neurosecretory signals, namely rhythmic secretion of melatonin (Wurtman et al, 1964; Reiter, 1980). In these species, such as the hamster or the sheep, administration of melatonin will mimick reproductive changes caused by short photoperiods in animals exposed to long photoperiods (Tamarkin et al, 1976; Arendt et al, 1983); in different conditions, melatonin can also act as a progonadal agent and provoke recrudescence of testes growth in animals exposed to short photoperiods (Hoffmann, 1979). At the present time, the only product of the pineal gland which appears to have a neuroendocrine function is melatonin, although a role has been suggested for other indoleamine derivatives such as 5-methoxytryptamine in specific animal species (Pévet, 1983).

MELATONIN AND SEXUAL DEVELOPMENT OF THE RAT

It has been difficult to assess the role of the pineal, and of melatonin, in species which were relatively insensitive to seasonal variations in the photoperiod. The laboratory rat is such a species. Only recently were complete studies on the effect of melatonin in the rat performed: these permitted to shed light on a possible role of melatonin during the pubertal development of the rat.

1. The male rat

The immature period of the male rat (20 to 40 days after birth) is characterized by markedly increased FSH secretion and enhanced pituitary GnRH receptor number (Aubert et al, 1982). In order to understand the role of melatonin in the male rat, melatonin was administered during different periods of sexual development. The effects of these injections were

tested on various parameters of the neuroendocrine-reproductive axis. The results (Lang et al, 1983) showed that melatonin had little effect when treatment occurred during the early prepubertal period (5-20 days of age) or in adult animals (70-90 days old), but it had a pronounced inhibitory influence when injected during the period of sexual development from 20 to 40 days of age (fig. 1 and fig. 2). This inhibiting effect of melatonin on sexual maturation in the male rat was found to be dose-dependent in a dose range from 5 to 100 g melatonin given daily in the late afternoon. If one considers the weights of testes and seminal vesicles and the concentration of plasma testosterone, which

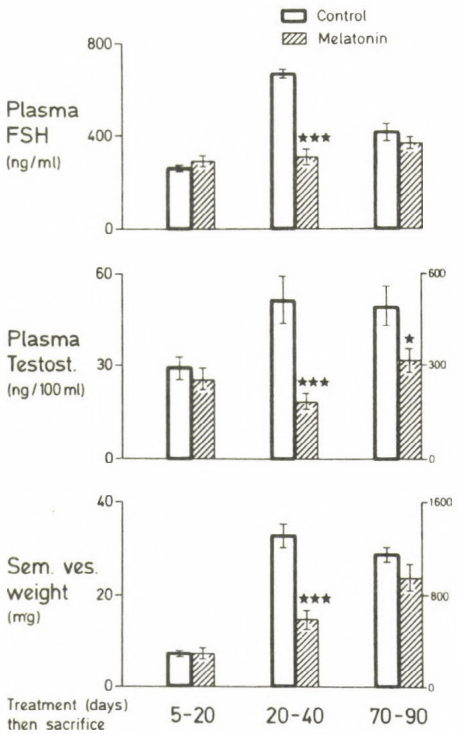

*Fig. 1 Influence of daily injections of melatonin on plasma FSH and testosterone levels and seminal vesicle weights of male rats during different stages of development. Groups of about 12 animals (▨) received daily injections (at 1700) of 100 µg melatonin during three different periods: 5-20 days (prepubertal age), 20-40 days (pubertal age), 70-90 days (age of adulthood). Control animals (□) received 100 µl physiological NaCl solution containing 10% ethanol. The rats were killed on the day after the last injection between 1000 and 1100 h. Plasma hormone levels and seminal vesicle weights are shown as the mean ± SE: ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$ (determined by t test). From Lang et al, 1983, with permission.*

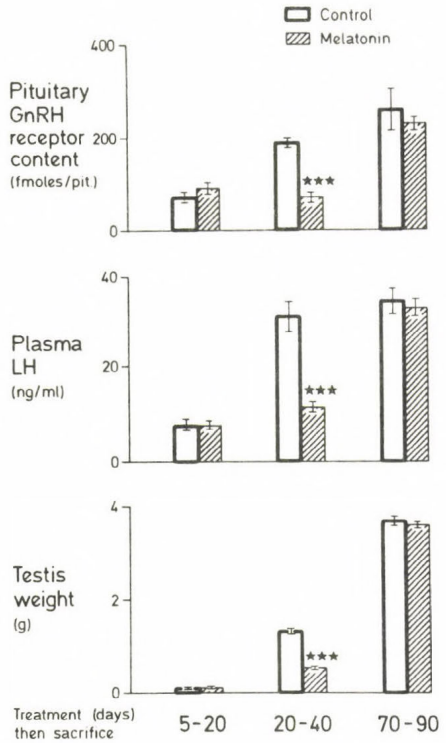

Fig. 2 Influence of daily injections of melatonin on pituitary GnRH receptors number, plasma LH concentration, and testis weight of male rats during different stages of development. Group of about 12 rats were treated as described in Fig. 1. Significance is explained in Fig. 1. From Lang et al, 1983, with permission.

are the most adequate parameters to follow sexual maturation, 5 g of melatonin given daily starting at 20 days was found to significantly decrease these parameters as seen at 45 days of age (Lang et al, 1983); 10 g daily was more active and a maximum effect was obtained with 100 g daily, as shown in figures 1 and 2.

These studies raised several questions which were further answered in our laboratory.

1. Is the time of injection of melatonin during the light-dark cycle of importance?

2. Does sexual maturation recover after cessation of melatonin administration?

3. Does prolonged melatonin administration maintain the state of delayed sexual maturation indefinitely?

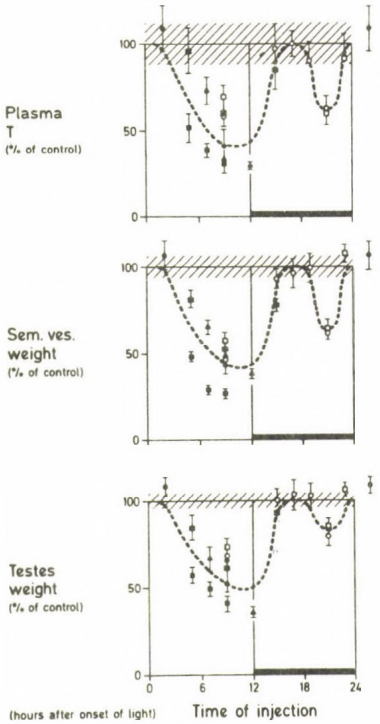

*Fig. 3 Effects of daily melatonin injections from 20 to 40 days of age given at different time during the day-night cycle on the mean weights of testes and seminal vesicles and on plasma testosterone concentration in the male rat. Control animals received 100 µl of saline containing 10% ethanol at 9 h after the onset of light. All data are expressed in percentage of the mean of control values, represented by the line on the top of the graph (± SEM: hatched area). The dotted line represents a suggested evolution of the inhibitory action of melatonin. Six series of rats each containing a control group, and different groups treated at different times during the day or night (10-12 animals/group) were studied at different times of the year and the mean values (± SEM) are represented as follows : ● = May, * = June, ▲ = August, ■ = December, ○ = February, □ = April. Closed symbols (●,*,▲,■) represent series with a light period from 0700 to 1900 h, open symbols (○,□) represent series with a light period from 1200 to 2400 h (○) and from 2400 to 1200 (□). All rats were maintained at a 12:12h light-dark schedule, as indicated at the bottom of the graph. From Lang et al, 1984a, with permission.*

In answer to the first question, it was shown that the time of administration of melatonin within the day-night cycle is critical for melatonin action. Immature male Wistar rats with a LD 12:12 light-dark cycle received daily melatonin

injections of 100 g s.c. from 20 to 40 days of age. Melatonin given during the late photophase (9h after the onset of light), or during the late scotophase (9h after the onset of darkness) inhibited growth of testes and seminal vesicles, lowered plasma levels of testosterone, LH and FSH, and decreased the number of pituitary GnRH receptors: as already stated, these observations reflect delayed sexual maturation at 40 days in the male rat (Lang et al, 1984a). In contrast, none of these effects was observed when melatonin was injected during the early photophase or scotophase (5h after the onset of light or darkness, respectively). In a more systematic study of the action of melatonin in relation to time of the day, it was found that the inhibitory influence of melatonin increased gradually during the late photophase and reached a peak just before the onset of darkness. The effect then disappeared during the first 7h in the dark phase; however, as already mentioned, melatonin injected 9h after the onset of darkness again had an inhibitory effect equivalent to about 50% of that observed in the late photophase, whereas administration 2h later remained without effect (fig. 3).

To answer question 2, chronic melatonin administration from day 20 to 45 of life only caused a temporary delay, and normal sexual maturation resumed after cessation of melatonin treatment (Lang et al, 1984b). In addition, to answer question 3, an escape phenomenon was present when melatonin was administered from day 20 until day 100 of life. By day 80, testicular and seminal vesicles weights, and plasma FSH and testosterone levels started to increase and reached adult levels by day 100 of age, in spite of persisting melatonin injections performed late in the afternoon (Lang et al, 1984b). Thus it appears that daily melatonin treatment of male rats delays rather than permanently inhibits sexual maturation.

2. The female rat

In the immature female rat, daily melatonin treatment starting at day 15 of age was also shown to delay sexual maturation. In this model, 100 g/day of melatonin delayed vaginal opening and disrupted the normal cyclicity of the first oestrous cycles: the cycles were irregular and less frequent (Rivest et al, 1984a). No variations of ovarian nor uterine weights were observed after opening of the vagina in melatonin-treated rats. Furthermore, although plasma levels of LH, FSH and estradiol were similar to those of control animals for samples taken during the diestrous phases, there was an enhanced proestrous surge of these hormones under melatonin treatment. In the pituitary, FSH concentrations were higher during diestrus and lower during proestrus. This hormonal pattern suggest a build-up phenomenon due to the low frequency of proestrous surges in melatonin-treated rats. Hence, the delayed vaginal opening and the infrequent proestrous surges could be one consequence of a single phenomenon, namely the lack of triggering signal for the increase in estradiol with its subsequent positive feedback on LH and FSH. This irregularity could be due to a direct action of melatonin on the pulsatile or basal release of GnRH, since

pituitary receptor number of GnRH were decreased after melatonin treatment. The greatest sensitivity to the delaying action of melatonin occurred when melatonin was given 11h after the onset of light. This was true for two lighting regimens tested, LD 12:12 and LD 16:08, suggesting that this window of sensitivity to melatonin is synchronized by the onset of light (Rivest et al, 1984a, 1984b). Furthermore, melatonin acted in the female only when it was given prior to vaginal opening: if treatment was started on the day of vaginal opening, it did not provoke irregular estrous cycles (Rivest et al, 1984c).

Thus, daily administration of melatonin to male and female rats delays sexual maturation. The site of action of melatonin seems to be at the hypothalamic level, probably on the secretion of GnRH since the number of GnRH receptors in the pituitary is lower after injections of melatonin in both sexes. It has been shown that the number of pituitary GnRH receptors is directly related to prior stimulation by GnRH. The period of sensitivity to administration of melatonin is dependent on the time of the day with maximal sensitivity present 10 to 12h after the onset of light; the time of appearance of this window of sensitivity is independent of the lighting regimen tested. Furthermore, melatonin acts only during pubertal development in a reversible manner, and treatment performed in adult animals has no visible effects neither in the male nor in the female.

SPECIFICITY OF MELATONIN

The ability of 7 different indoleamines or indoleamine derivatives to delay sexual maturation of male rats was studied. Of the 7 compounds tested, only 5-methoxytryptamine and 6-hydroxymelatonin in addition to melatonin were found to inhibit the neuroendocrine-reproductive axis. The potencies of these two compounds when injected in the afternoon were about 1/10 that of melatonin. Thin layer chromatographic analysis of plasma extracts from rats injected from 20 to 40 days of age with **5-methoxytryptamine** and sacrificed shortly after the last injection showed that part of the injected 5-methoxytryptamine had been acetylated to melatonin (Lang et al, 1984c). Therefore, it cannot be excluded that the inhibitory action of 5-methoxytryptamine on sexual maturation might be due entirely to its enzymatic transformation into melatonin. Serotonin, N-acetylserotonin, 5-methoxytryptophol and 5-hydroxytryptophol had no significant effect on sexual maturation. These results suggest that melatonin is the principal pineal indoleamine which is responsible for the timing of sexual maturation in the rat.

PINEAL GLAND AND HUMAN PUBERTY

In the human as well as in the animal, melatonin is secreted in a cyclic fashion. Melatonin is high during the night in plasma, cerebrospinal fluid, and in urine (Arendt et al, 1977; Lang et al, 1981a).

Tumors of the pineal region have been observed in association with precocious puberty (Kitay, 1954), but it is difficult to assess a role to the pineal since the majority of the tumors are not pinealocytomas but dysgerminomas secreting hCG.

Most of the data published on serum melatonin levels in prepubertal and pubertal subjects consist of observations made on plasma samples collected during **daytime** (Silman et al, 1979; Tamarkin et al, 1982; Ehrenkranz et al, 1982; Lenko et al, 1982). With one exception (Silman et al, 1979), these studies found no difference between melatonin concentrations in prepubertal children and adults. Studies which also measured **nocturnal** melatonin proved more fruitful. Recently,

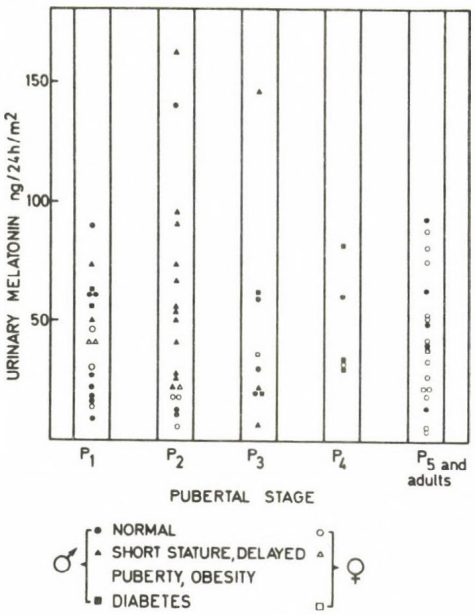

Fig. 4 Urinary excretion of melatonin per 24 hours in 43 children of both sexes from 5 to 17 years. Normal as well as obese children, or children with delayed puberty, short stature or insulin-dependent diabetes were studied. No variation of melatonin excretion was found between the different stages of puberty (P1 = No sign or puberty, P5 = adulthood). With permission, from Lang et al, 1984d, Williams and Wilkins. The Control of Onset of Puberty II (Stresa Conference 1981). M.M. Grumbach, P.C. Sizonenko and M.L. Aubert (eds). In press.

Waldhauser et al (1984) found a difference on midnight levels of melatonin between prepubertal children less than 7 years old and prepubertal and pubertal subjects over 7 years of age. In two studies (Ehrenkranz et al, 1982; Tamarkin et al, 1982) nighttime melatonin levels of prepubertal children were compared with those of adults. Concentrations tended to be higher before puberty, but the differences noted were not statistically significant. However, two groups (Gupta et al, 1983; Attanasio et al, 1983) did find a decrease in the difference between nighttime and daytime serum level of melatonin during sexual maturation.

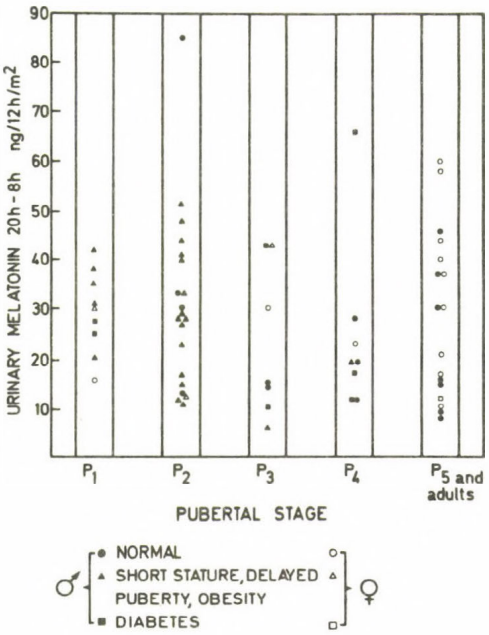

Fig. 5 *Night urinary (20h-08h) excretion of melatonin in the same children as in fig. 4. For symbols see legend fig. 4. From Lang et al, 1984d, Williams and Wilkins. The Onset of Puberty II (Stresa Conference 1981). M.M. Grumbach, P.C. Sizonenko and M.L. Aubert (eds). In press.*

Measures of melatonin in the urine can provide an integrated picture of the total amount of melatonin produced. Urinary excretion of melatonin in 43 children at different stages of puberty was measured either during the night period (8 p.m. to 8 a.m.) or during a 24 hour period (Sizonenko et al, 1982). Although a great variation in urinary excretion was observed (6.4 to 161 ng/24h/m2), no difference between the sexes and the stages of puberty was observed for melatonin excreted during 24h (fig. 4) nor for melatonin excreted specifically during the night (fig.5).

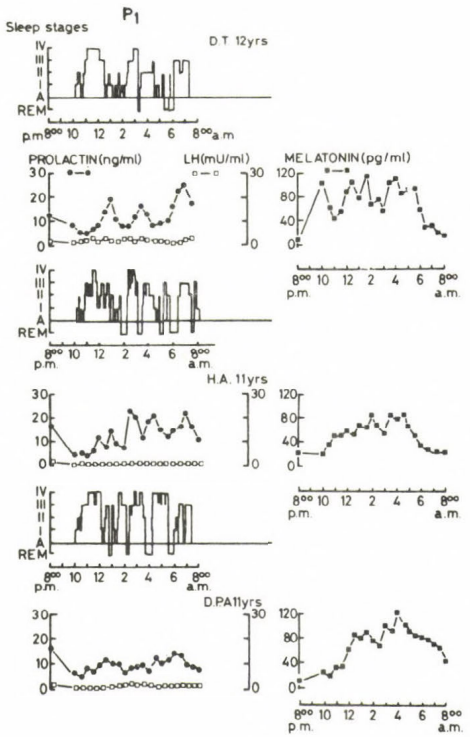

Fig. 6 *Plasma concentration of prolactin, LH and melatonin during sleep in 3 prepubertal boys (Stage P1). EEG recording was made simultaneously. A: awake. REM : rapid-eye movements. Reproduced with permission from Lang et al, 1984d. The Control of Onset of Puberty II (Stresa Conference 1981). M.M. Grumbach, P.C. Sizonenko, M.L. Aubert (eds). In press.*

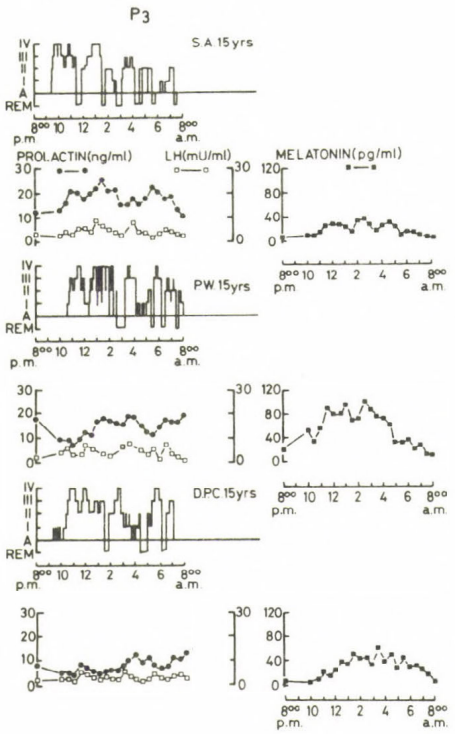

Fig. 7 Plasma concentration of prolactin, LH and melatonin during sleep in 3 mid-pubertal boys (Stage P3). For symbols, see legend fig. 6. Reproduced with permission from Lang et al, 1984d. Williams and Wilkins. The Control of the Onset of Puberty II (Stresa Conference 1982). M.M. Grumbach, P.C. Sizonenko and M.L. Aubert (eds). In press.

In a subsequent study, the nocturnal pattern of melatonin was compared between pre-puberty and post-puberty. Nine healthyboys were studied throughout the night: 3 were pre-pubertal (stage P1), 3 taken at mid-puberty (stage P3) and 3 at the end of pubertal period (stage P5). Blood samples were obtained every 30 minutes for determinations of melatonin, LH and prolactin concentrations. Sleep EEG was also monitored. Results showed that the secretory pattern of melatonin during the night is not different for the three stages of puberty studied. In contrast, nocturnal plasma

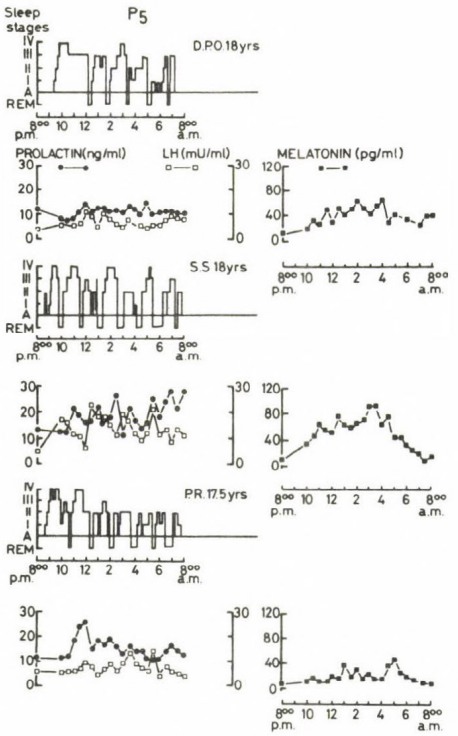

Fig. 8 *Plasma concentration of prolactin, LH and melatonin during sleep in 3 boys near adulthood (Stage P5). For symbols, see legend of fig. 6. Reproduced with permission from Lang et al, 1984d. Williams and Wilkins. The Control of the Onset of Puberty II (Stresa Conference 1981). M.M. Grumbach, P.C. Sizonenko and M.L. Aubert (eds). In press.*

concentrations of LH increased steadily during these stages of sexual development (fig. 6 to 8). One group, on the other hand, has reported increasing excretion of melatonin in the urine during puberty (Penny, 1982).

It is difficult from the work presented and cited to attribute a role to the pineal gland during human pubertal development. Measurements of urinary melatonin metabolites such as conjugated 6-hydpoxymelatmnin did not show a correlation between daily exaretion rates and age or pubertal stages, except for an increase mbserved at the time of onset of breast developement (Tanner stage II), No difderelce was seen during puberty in boys. The significance of this difference in Tanner II/girls is not known (Tetsuo et al, 1982). Results obtained in studies on different pathologies offered no definite conclusion. In boys with delayed puberty, Cohen et al (1982) measured higher daytime melatonin levels

than in a control population. The pubertal melatonin pattern of these subjects fell after mid-puberty. Tamarkin et al (1982) did not find abnormal changes of plasma melatonin in obese patients and subjects with Prader-Willi's syndrome. In contrast, Ehrenkranz et al (1982) reported normal daily rhythm of plasma melatonin in precocious puberty.

These seemingly discordant findings do not completely rule out an action of melatonin during human pubertal development. It is not excluded that melatonin induces subtle rather than gross changes in gonadotropin secretion which cannot be defined because of poor means of investigations (for example, a change in the pulsatile pattern). In addition, as is shown in animals, melatonin is active only at a certain time during the day or the night. If this is also true for the human, it renders manystudies particularly difficult. It is also possible that a rhythmic and life-long modulation of melatonin receptors or target tissue receptivity to melatonin action takes place. Melatonin receptors have been identified (Cardinali et al, 1979; Cohen et al, 1978; Lang et al, 1981b) and the sensitivity of such receptors may change during pubertal development. Thus, serum levels of melatonin might not provide as much information as measurements of melatonin receptors on target tissues.

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REFERENCES

ARENDT J, WETTERBERG L, HEYDEN, T, SIZONENKO, PC, PAUNIER, L (1977) Radioimmunoassay of melatonin: human serum and cerebrospinal fluid. Hormone Research 8:65-75

ARENDT J, SYMONS AM, LAND CA, PRYDE SJ (1983) Melatonin can induce early onset of the breeding season in ewes. J Endocr 97:395-400

ATTANASIO A, BORRELLI P, MARINI R, CAMBIASO P, CAPPA M, GUPTA D (1983) Serum melatonin in children with early and delayed puberty. Neuroendocr Lett 5:387-392

AUBERT ML, LANG U, NAWRATIL MF, BRADTKE J, SIZONENKO PC (1982) Ontogenesis of pituitary receptor sites for Gonadotropin Releasing Hormone (GNRH) in male and female rats. In: "Ontogenesis of the Endocrine system" (Saez JM, Bertrand J, Ducharme JR, Collu R, eds), Les Editions INSERM 110:319-34

CARDINALI DP, VACAS MI, BOYER EE (1979) Specific binding of melatonin in bovine brain. Endocrinology 105:437-441

COHEN HN, ROSELIE D, CHABNER B, SCHMIDT TJ, LIPPMANN M (1978) Evidence for a cytoplasmic melatonin receptor. Nature 274:894-895

COHEN HN, HAY JD, ANNESLEY TM, BEATSALL GH, WALLACE AM, SPOONER R, THOMSON JA, EASTWOLD P, KLEE GG (1982) Serum immunoreactive melatonin in boys with delayed puberty. Clin Endocrinol 17:517-521

EHRENKRANZ JR, TAMARKIN L, COMITE F, JOHNSONBAUGH RE, BYBEE DE, LORIAUX DL, CUTLER GB (1982) Daily rhythm of plasma melatonin in normal and precocious puberty. J Clin Endocr Metab 55:307-310

GUPTA D, RIEDEL L, FRICK HJ, ATTANASIO A, RANKE MB (1983) Circulating melatonin in children in relation to puberty, endocrine disorders, functional tests and racial origin. Neuroendocr Lett 5:63-78

HOFFMANN K (1979) Photoperiod, pineal melatonin and reproduction in hamsters. Prog. Brain Res 52:397-415

KITAY JI (1954) Pineal lesions and precocious puberty: a review. J Clin Endocr Metab 14:622-625

LANG U, KORNEMARK M, AUBERT ML, PAUNIER L, SIZONENKO PC (1981a) Radioimmunological determination of urinary melatonin in human: correlation with plasma levels and typical 24 hour rhythmicity. J Clin Endocr Metab 53:645-650

LANG U, AUBERT ML, SIZONENKO PC (1981b) Location of melatonin receptors. Pediatr Res 15:80 (Abstract)

LANG U, AUBERT ML, CONNE BS, BRADTKE JC, SIZONENKO PC (1983) Influence of exogenous melatonin on melatonin secretion and on the neuroendocrine reproductive axis of intact male rats during sexual maturation. Endocrinology 112:1578-1584

LANG U, RIVEST RW, SCHLAEPFER LV, AUBERT ML, SIZONENKO PC (1984a) Diurnal rhythm of melatonin action on sexual maturation of the male rats. Neuroendocrinology 38:261-268

LANG U, AUBERT ML, RIVEST RW, VINAS-BRADTKE JC, SIZONENKO PC (1984b) Daily afternoon administration of melatonin does not irreversibly inhibit sexual maturation in male rat. Endocrinology (in press)

LANG U, RIVEST RW, AUBERT ML, VINAS JC, SIZONENKO PC (1984c) Inhibitory action of 5-methoxytryptamine on sexual maturation of male rats might be due to enzymatic conversion to melatonin. 3rd Colloquium of the European Pineal Study Group, Pécs, (H)13-17 August (Abstract)

LANG U, LENKO HL, BRADTKE JC, DELAVY B, AUBERT ML, SIZONENKO PC (1984d) Pineal gland and puberty. In: "The control of the onset of puberty II" (Stresa Conference 1981). Grumbach MM, Sizonenko PC, Aubert ML, eds. Williams and Wilkins, in press.

LENKO HL, LANG U, AUBERT ML, PAUNIER L, SIZONENKO PC (1982) Hormonal changes in puberty. VII. Lack of variation of daytime plasma melatonin. J Clin Endocr Metab 54:1056-1058

PENNY R (1982) Melatonin excretion in normal males and females: increase during puberty. Metabolism 31:816-823

PEVET P (1983) Is 5-methoxytryptamine a pineal hormone? Psychoneuroendocrinology 8:61-73

REITER RJ (1980) The Pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1:109-131

RIVEST RW, LANG U, AUBERT ML, SIZONENKO PC (1984a) Daily administration of melatonin delays vaginal opening and disrupts the first estrous cycles: Evidence that these effects are synchronized by the onset of light. Endocrinology (in press)

RIVEST RW, LANG U, AUBERT ML, NAWRATIL MF, SCHERRER A, SIZONENKO PC (1984b) The use of two different lighting regimens allows the dissociation of the mechanisms responsible for the action of melatonin on estrous cycles and those provoking the proestrous surge of gonadotropins. Ann Rev Chronopharmacol 1 (in press)

RIVEST RW, LANG U, AUBERT ML, SIZONENKO PC (1984c) The disrupting effect of melatonin on the estrous cycle of rats is a developmental rather than an acute effect. 3rd. Colloquium of the European Pineal Study Group, Pécs (H), 13-17 August (Abstract)

SILMAN RE, LEONE RM, HOOPER RJL, PREECE MA (1979) Melatonin, the pineal gland and human puberty. Nature 282:301-303

SIZONENKO PC, LANG U, AUBERT ML (1982) Neuro-endocrinologie de la puberté. Rôle de la mélatonine chez l'homme. Annal Endocr 43:453-464

TAMARKIN L, WESTROM WK, HAMILL AI, GOLDMANN BD (1976) Effect of melatonin on the reproductive systems of male and female Syrian hamsters: A diurnal rhythm in sensitivity to melatonin. Endocrinology 99:1534-1541.

TAMARKIN L, ABASTILLAS P, CHEN HC, MCNEMAR A, SIDBURY JB (1982) The daily profile of plasma melatonin in obese and Prader-Willi syndrome children. J Clin Endocr Metab 55:491-495

TETSUO M, POTH M, MARKEY SP (1982) Melatonin metabolite excretion during childhood and puberty. J Clin Endocr Metab 55:311-313

WALDHAUSER F, WEISZENBACHER G, FRISCH H, ZEITLHUBER U, WALDHAUSER M, WURTMAN RJ (1984) Fall in nocturnal serum melatonin during prepuberty and pubescence. The Lancet I:362-365

WURTMAN RJ, AXELROD J, FISHER JE (1964) Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. Science 143:1328-1330

The Pineal Gland
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THE HUMAN PINEAL GLAND: MELATONIN AS A TOOL IN THE DIAGNOSTICS OF ENDOCRINE AND MENTAL DISEASES

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INTRODUCTION

This review will mainly cover recent studies about melatonin as a diagnostic tool in psychiatry and endocrinology. For background information about the pineal gland in general the reader may consult books edited by Oksche and Pévet (1981), Reiter (1982), Relkin (1983) and Axelrod, Fraschini and Velo (1983). Two broad reviews with more than hundred references about the pineal gland hormone melatonin in humans were recently published by Vaughan (1984) and Waldhauser et al. (1984a). The evaluation of the research included studies of melatonin in biological material, factors associated with variation in melatonin secretion and administration of melatonin.

The present review gives further support to a possible relationship between the pineal-hypothalamic-pituitary-adrenal network and other structures interacting with this network. There is reason to believe that the interaction found in animal as well as in in vitro experiments between the pineal gland and the hypothalamic-pituitary-adrenal axis may also to some extent be present in man.

Genetic regulation of melatonin

It is well established that the production of melatonin varies between individuals but the reasons for this variation is not known. In one study (Wetterberg et al. 1983) twenty-three complete nuclear families comprising 107 individuals free of medication were examined for their urinary excretion of melatonin during the night. The genetic analysis did not show any evidence in favour of a bi- or trimodal distribution of the melatonin values. Evidence was obtained for a major locus influencing the primary melatonin concentrations. The study has shown that melatonin excretion in the urine is likely to be under genetic control.

Serum melatonin in relation to light influx, season, height, sex, and age

In addition to the possible genetic traits controlling the basal levels of serum and urine melatonin, several environmental factors modulate the levels. Melatonin secretion in man displays a circadian rhythm with the lowest

concentrations during the day and the peak levels during the night at about 02 h (Arendt et al. 1977). Bright light at night has been shown to suppress the serum levels of melatonin (Lewy et al. 1980). The use of eye-glasses has also been reported to be associated with lower levels of melatonin (Erikson et al.1983).

A seasonal variation has been reported in morning serum melatonin occurrence, with lower levels during the spring-summer period compared to the winter samples (Beck-Friis et al. 1984).

Arendt et al. (1982) reported a significant positive correlation between melatonin and body weight and a significant negative correlation between melatonin and obesity index. In a sample of 87 individuals the maximum nocturnal serum melatonin level was found to correlate negatively to body height (Beck-Friis et al. 1984a). In the same sample there was no difference in melatonin levels between 39 females compared to 48 males. A significant age-dependent reduction in serum melatonin concentration in human subjects has been reporterd by Touitou et al. (1981, 1984), Iguchi et al.(1982a) as well as Beck-Friis et al. (1984a). The variations of urinary melatonin excretion in humans during the first 30 years of life was studied by Lemaitre et al. (1981). They found high urinary melatonin in newborns and a decrease the first year of life. Melatonin increased in children and decreased in adults.

Melatonin and puberty in humans

An interesting set of reports has triggered a discussion about the possible role of melatonin during puberty. Lenko et al. (1982) reported a lack of variation of daytime plasma melatonin in puberty.

Sizonenko et al. (1982) concluded that the studies on night and day plasma concentrations and urinary excretions of melatonin did not attribute a role to melatonin during human pubertal development. Attanasio et al. (1983), however, reported that the day-night increment in melatonin levels was related to the maturational stage. The subjects with precocious puberty had smaller increments than the aged matched controls.

Waldhauser et al. (1984) reported data interpreted as evidence for a relationship between a decrease in serum melatonin levels and the development of puberty. This paper in the Lancet led to a review article in Science under the title "Puberty mystery solved" (20 Jan., p. 272). A comment to this article by Klein (1984) states that neither melatonin nor pinealectomy has been experimentally shown to either trigger or block puberty and that the possible importance of the pineal gland controlling puberty is not clearly established. Obviously more work remains to be done before the possible role of melatonin in puberty in humans is clarified.

Penny and Goebelsmann (1984) found that estradiol valerate given to a normal adult male significantly decreased mean plasma melatonin levels. The authors conclude that the decrease in melatonin levels post estradiol is the reverse of the response expected based on animal experimental data. Thus the functional role of the pineal gland in animal and in man may well differ and the results from animal studies must be interpreted cautiously.

Melatonin secretion in patients with pituitary disease

An alteration of the serum melatonin pattern has been reported to occur in Cushing's syndrome (Werner et al. 1981; Fèvre-Montange et al. 1983).

In another study on the possible relation between cortisol and melatonin the metyrapone test was used by Brismar et al. (1982). The melatonin secretion increased significantly in eight out of ten patients during and in one after the metyrapone administration. The results were interpreted as a possible relationship between melatonin and ACTH-cortisol secretion, possibly through a suprapituitary interaction.

To further elucidate the relation between melatonin and the pituitary-adrenal activity Brismar et al. (1984) studied the circadian rhythmicity of serum melatonin, ACTH and cortisol and the urinary excretion of melatonin, cortisol and Porter-Silber chromogens before, during and after the administration of 750 mg metyrapone every four hours during 24 hours in two healthy volunteers and in two patients with asymptomatic hyperparathyroidism and a prolactin secreting adenoma. Also in this study the excretion of melatonin increased during the administration of metyrapone. The excretory maximum of melatonin preceeded the maximal ACTH-adrenal response. Serum melatonin remained unchanged during the administration of metyrapone.The night after the metyrapone test serum melatonin was depressed in the patients with the most pronounced ACTH-cortisol response following the test. The finding indicates that melatonin is suppressed when ACTH-cortisol excretions is increased.

In the same study of Brismar et al. (1984) it was shown that serum melatonin increased by the metyrapone induced corstisol inhibition while high cortisol levels were accompanied by reduced serum melatonin levels. Hypercortisolism by itself might modify the melatonin levels.

The metyrapone test may also influence the liver and kidney clearance. It may thus be of interest to further study the effect of disturbance in metabolic clearance . in particular since Iguchi et al. (1982b) reported elevated serum melatonin levels in daytime in patients with liver cirrhosis. The authors interpret their findings as possibly due to decreased clearance rate, lowered activity of 6 β -hydroxylase, and competition with bilirubin in the intrahepatic transport system.

Melatonin in primary hyperparathyroidism

As will be reported below melatonin may be a marker for a subtype of affective disorders. Affective symptoms often accompany primary hyperparathyroidism. It was thus of interest to study the role of the pineal gland in the regulation of the parathyroid hormone (PTH) secretion. Brismar et al. (1984b) examined the circadian secretion of melatonin in primary hyperparathyroidism before and after operation. After the removal of a parathyroid adenoma all patients became normocalcemic and serum melatonin levels diminished significantly while the circadian pattern did not change. The preoperative melatonin levels were high and normalized after the removal of the PTH-producing adenomas.

The reason for the higher melatonin levels preoperatively may be related to the higher circulating calcium concentrations affecting the syntheses of melatonin in the pineal gland in accordance with in vitro studies.

In the same patients the serum cortisol levels decreased when the melatonin secretion diminished which indicates that the melatonin-cortisol interaction is more complex than a regulatory mechanism by a single feedback loop.

Melatonin secretion in relation to cortisol and ACTH in psychiatric patients - low melatonin in depression

The interaction between the pineal gland and the pituitary and adrenal glands seems to be of a complex nature. In a neuroendocrine investigation of patients with major depressive disorder Beck-Friis et al. (1984b) evaluated the pathology of the hypothalamic-pituitary-adrenal axis and the function of the pineal gland as revealed by the serum melatonin levels. In all 87 individuals were examined. The 24-hour profiles of melatonin and cortisol in serum, morning levels of ACTH in plasma and the dexamethasone suppression test were investigated in patients with acute depression as well as in patients with major depressive disorder studied in remission.

In this study there are several findings giving further indications of a functional relationship between the pineal gland and the hypothalamic-pituitary-adrenal axis. There was a significant decrease of the maximum nocturnal melatonin level in the acutely ill depressed patients with abnormal dexamethasone suppression test compared to those with normal test as well as to control subjects. The maximum nocturnal melatonin levels were unaltered when the same patients were reinvestigated in a state of clinical remission.

A significant decrease of melatonin after dexamethasone was seen at 08 h in the unipolar-bipolar patients in remission as well as in the healthy subjects, but not in the acutely ill depressed patients in relapse. Melatonin is postulated to be an inhibiting factor for the hypothalamic corticotrophin releasing factor during depression.

The low nocturnal melatonin level was proposed to be a trait marker for major depressive disorder and depressive states with abnormalities in the hypothalamic-pituitary-adrenal axis. This abnormality has been proposed to be due to a hypersecretion of the corticotrophin releasing factor with a subsequent stimulus-induced pituitary desensitization.

Interestingly, a tendency to a phase advance of the melatonin peak was seen in the acutely ill depressed patients with abnormal dexamethasone suppression test pointing to a possible involvement of the suprachiasmatic nuclei in the hypothalamus.

Several authors have reported melatonin levels to be lower in some types of depressive disorders (Wetterberg et al. 1979; Mendlewicz et al. 1979; Wirz-Justice and Arendt 1979; Beck-Friis et al. 1984). Wetterberg et al. (1984) proposed low nocturnal melatonin levels to be a "trait dependent" marker for a subtype of major depressive disorder. The specificity of low melatonin as a marker for affective disorders versus other psychiatric conditions is however unknown.

Ferrier et al. (1982) have reported low levels of melatonin also in schizophrenic patients, although the findings might be due to a possible inclusion of patients with affective symptoms (Wetterberg et al. 1982). In a study of Beck-Friis et al. (1984c) a relationship between nocturnal melatonin levels and clinical variables was tested. The study included a control group of 33 healthy subjects. A potentially interesting finding was that depressed patients with parental loss before 17 years of age, had subnormal levels of melatonin thus differing from patients with no parental loss. A possible explanation for this finding might be that the stress factor "parental loss" might cause an increased steroid secretion which at a certain developmental stage permanently alters the receptor sensitivity in the pineal gland, responsible for melatonin production.

In animal experiments, Yuwiler and Brammer (1981) found that neonatal hydrocortisone treatment affected the maturation in the pineal noradrenergic system. This experimental condition may resemble early stress reaction in man.

A significant regression was found between the maximum nocturnal melatonin levels and the clinical ratings as well as some biochemical measures. The conclusions were that there might be a low melatonin syndrome in depression characterized by low nocturnal melatonin, abnormal dexamethasone suppression test, disturbed 24-hour rhythm of cortisol and less pronounced daily and annual cyclic variation in depressive symptomatology (Beck-Friis and Wetterberg, 1984, Beck-Friis et al. 1984).

The hypothesis of a functional relationship between low melatonin and a subtype of depressive disorder should be tested for its validity in other clinical trials. Also, the deminished rhythmicity found in depressed patients with low nocturnal melatonin may also, in principle, be present together with affective symptoms in other diagnostic groups.

Melatonin in schizophrenic patients

Beckmann et al. (1984) investigated the melatonin concentration in cerebrospinal fluid from 15 medicated and 13 unmedicated paranoid schizophrenic patients as well as 16 healthy controls. The cerebrospinal fluid, when sampled in the morning and the melatonin levels were low, about 0.06 nmol/l (15 pg/ml) in all three groups. No significant correlations were found between melatonin concentrations and various other biochemical substances such as noradrenalin, cyclic adenosine 3',5'-monophosphate, prolactin and cortisol. The results must be interpreted with caution since a change in the biological rhythm cannot be determined with a single sample. Also, the low morning values of melatonin will not necessarily reflect possible nocturnal amplitude alterations.

As mentioned above Ferrier et al. (1982) reported low levels of plasma melatonin in schizophrenic patients.

Hanssen and Wetterberg (1983) reported about chronic schizophrenic patients with nocturnal melatonin levels above the mean of controls. These patients improved clinically when treated with propranolol. The authors hypothesized

that there might be a subgroup of schizophrenic patients with a higher than normal noradrenergic receptor activity. This activity might be revealed by the high melatonin production. High doses of propranolol markedly lowered the melatonin levels. When a phenothiazine drug was added to the propranolol treatment the hormonal levels remained low.

Cremer-Bartels et al. (1983) reported that schizophrenic patients might have lower levels of an inhibitor substance of hydroxy-indole-O-methyltransferase (HIOMT)in their blood serum. The identification of the low molecular weight fraction is of great interest since Cremer-Bartels et al. (1983) postulate a possible imbalance of inhibitors of HIOMT might be involved in schizophrenia.

For many years an association between pineal gland metabolism and mental disorders, particularly in schizophrenia has been suggested. Smith et al. (1981) reported a significant increase in HIOMT in the pineal glands from schizophrenics. However, Owen et al. (1983) found no difference in the enzyme activity in the pineal from schizophrenic patients compared to controls.

There is also a hypothetical link between schizophrenia and the opioid peptide system, in particular des-tyr-gamma-endorphin, which has been reported to have an antipsychotic effect in some schizophrenic patients (Verhoeven et al. 1984).

It is thus interesting that Claustrat et al. (1981) found a rise in urinary melatonin after stimulation by des-tyr-gamma-endorphin. On the other hand beta-endorphin levels decreased significantly after i.m. injections of melatonin to 8 healthy subjects (Lissoni et al. 1984). The results indicate that the pineal gland may have an inhibitory effect on the opioid system, while the opioid peptides seem to stimulate melatonin secretion, at least under some conditions. The effect of the pineal gland on the pituitary could be direct or mediated via the hypothalamic level.

Melatonin in alcoholic patients

Borg et al. (1983) reported lower levels of urinary melatonin in alcoholic patients compared to controls. The melatonin levels of teetotallers were higher than among subjects with an alcohol consumption. Studies of the main noradrenaline metabolite in the central nervous system, 3-methoxy-4-hydroxyphenyl-ethylenaglycol in the cerebrospinal fluid have indicated a connection between alcohol intoxication and central noradrenaline metabolism. Melatonin may be considered an additional marker reflecting central noradrenergic mechanism. The possible relation of melatonin concentrations and noradrenalin metabolites may reveal new aspects of noradrenergic activity in alcoholics. The results of the study of Borg et al. (1983) are in line with the hypothesis of a low central noradrenergic activity during some phases of alcoholism.

Melatonin co-varies with fatigue and sleep/wake cycle

In a study by Akerstedt et al. (1982) melatonin excretion was studied in relation to subjective arousal during 64 h of sleep deprivation in 12 healthy males. The sleep-rated fatigue exhibited a circadian variation with the peak fatigue coinciding temporally with the peak excretion levels of melatonin.

In an experimental study, also of 12 healthy volunteers, Arendt et al. (1984) investigated the effects of chronic doses of 2 mg melatonin taken orally at 17.00 h for a period of four weeks. The self-rated tiredness was significantly increased in the evening during melatonin treatment. The authors found a slow onset of the increased evening tiredness which is more consistent with an effect on the timing mechanisms of sleep than an acute sleep-inducing effect.

Bratlid et al. (1984) recently reported on the effect of exposure to bright artificial light on melatonin secretion and "mid-winter insomnia" in northern Norway. In 17 individuals exposed to more than 2,000 lux for 30 min between 07.30 and 08.30 successively for 5 days in the winter season the serum melatonin levels increased in the evening sampling period. This was interpreted as a phase advance due to the light stimulation which entrained the sleep/wake cycle to a more normal 24-hour pattern.

Melatonin in cluster headache

Cluster headache is characterized by regular attacks often appearing at night time after one or two hours of sleep. Melatonin levels seem to be lower at 02 h during active cluster periods than during clinical remission (Waldenlind et al. 1984). This finding may reflect an altered regulation of central neurotransmission in cluster headache.

Interference in the radioimmunoassay of melatonin

Some plastic material has been shown to interfer with the radioimmunoassay (RIA) for melatonin. The substances have been identified as tri-(2-butoxyethyl) phosphate (Beck et al. 1983) and dimethyl phtalate (Wetterberg et al. 1984b). The subsequent testing of authentic dimethyl phtalate reacted with the antiserum for the melatonin in the commercially available melatonin RIA by KALAB, Box 634, Danville. CA 94526, USA.

The described interfering compounds are used in manifacturing various plastic products such as pipettes, tubings and test tubes. In order to avoid interference in the RIA it is advisable to use glass material when collecting blood for serum or plasma and assaying melatonin.

Future developments of melatonin as a diagnostic tool in endocrinology and psychiatry

The present results indicate a possible relationship between the pineal-hypothalamus-pituitary-adrenal network. It is likely that other structures interact with this network. The circadian rhythm of melatonin may provide a possibility to measure a central biological clock mechanism and to test the influence of different environmental factors of the neuroendocrine system.

It is obvious that in addition to the genetic control of melatonin production, a host of environmental factors influence the secretion of melatonin in humans. Future studies will deal with the molecular-chromosmal bases for the production and regulation of the melatonin producing enzymes as well as the rhythmical variation in the sensitivity of the pineal to different endogenous and external signals.

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REFERENCES

Akerstedt. T., Gillberg. M. and Wetterberg. L. (1982). The circadian covariation of fatigue and urinary melatonin. Biol. Psychiatr. 17, 547-554.

Arendt. J., Wetterberg, L., Heyden. T., Sizonenko, P.C. and Paunier, L.(1977). Radioimmunoassay of melatonin: Human serum and cerebrospinal fluid. Hormone Res 8, 65-75.

Arendt. J., Hampton, S., English. J., Kwasowski. P. and Marks. V. (1982). 24-hour profiles of melatonin, cortisol, insulin, C-peptide and gip following a meal and subsequent fasting. Clin Endocrinol 16, 89-95.

Arendt, J., Borbely, A.A., Franey, C. and Wright, J. (1984). The effects of chronic, small doses of melatonin given in the late afternoon on fatigue in man: a preliminary study. Neurosci Lett 45, 317-321.

Attanasio. A., Borrelli, P., Marini, R., Cambiaso. P., Cappa. M. and Gupta. D.(1983). Serum melatonin in children with early and delayed puberty. Neuroendocrinol Lett 5. 387.

Beck. O., Friberg. Y., Johansson, L., Sääf. J., Waldenlind. E. and Wetterberg. L. (1983). Interference with the radio-immuno assay of melatonin by a substance(s) in vacutainer serum separation tubes (SST). Neuroendocrinol Lett 5, 405.

Beck-Friis, J, von Rosen, D., Kjellman, B.F., Ljunggren, J.-G.and Wetterberg,L. (1984a). Melatonin in relation to body measures, sex. age. season and the use of drugs in patients with major affective disorders and healthy subjects. Psychoneuroendocrinology. 9, 261-277.

Beck-Friis. J., Ljunggren, J-G., Thorén. M., von Rosen. D., Kjellman, B.F. and Wetterberg, L. (1984b). Melatonin, cortisol and ACTH in patients with major depressive disorder and healthy humans with special reference to the outcome of the dexamethasone suppression test. Psychoneuroendocrinology. In press.

Beck-Friis, J., Kjellman, B.F., Aperia, B., Undén, F., von Rosen. D., Ljunggren. J.-G. and Wetterberg, L. (1984c). Serum melatonin in relation to clinical variables in patients with major depressive disorder and a hypothesis of a low melatonin syndrome. Acta Psychiatr Scand. In press.

Beckmann, H., Wetterberg. L. and Gattaz. W.F. (1984).Melatonin immunoreactivity in cerebrospinal fluid of schizophrenic patients and healthy controls. Psychiatry Res 11, 107-110.

Bratlid. T., Hansen. T. and Linajaerde. O. (1984). The effect of morning exposure to bright artificial light on melatonin secretion and "midwinter insomnia" in northern Norway. XVth International Congress of ISPNE, Vienna. Abstract. 24.

Borg, S., Mossberg, D. and Wetterberg, L. (1983). Low urine melatonin concentration in alcoholics - a possible marker of a low central noradrenaline metabolism. Neuroendocrinol Lett 5, 411.

Brismar, K., Werner, S and Wetterberg, L. (1982). Melatonin and corticosteroid response to metyrapone in patients with pituitary disease. In The pineal and its hormones, Reiter. R.J. (Ed.) Alan R. Liss. Inc. New York. 283-292.

Brismar, K., Werner, S., Thorén, M. and Wetterberg, L. (1984). Metyrapone - a stimulatory test agent for melatonin as well as ACTH and cortisol secretion. J of Endocrinological Investigation. In press.

Brismar, K., Werner, S. and Wetterberg, L. (1984b). Pituitary and pineal function pre- and postoperatively in primary hyperparathyroidism. XXII Congress of Endocrinology. Quebec. 494.

Claustrat. B., Chazot. G., Brun. J. (1981). Melatonin secretion in man: Stimulating effect of destyrosine gamma endorphin. Neuroendocrinol Lett 3, 35-37.

Cremer-Bartels, G., Kuchle, H.J. and Rolfes. H. (1983). Inhibcition of hydroxy-indole-O-methyltransferase (HIOMT) by low molecular weight fractions (LWF). Neuroendocrinol Lett 5, 406.

Erikson, C., Kuller, R. and Wetterberg. L. (1983). Non visual effects of light. Neuroendocrinol Lett 5, 412.

Ferrier, I.N., Johnstone. E.C., Crow, T.J. and Arendt. J. (1982). Melatonin/cortisol ratio in psychiatric illness. Lancet i, 1070.

Fèvre-Montange, M., Tourniaire. J., Estour. B. and Bajard. L. (1983). 24 hour melatonin secretory pattern in Cushing's syndrome. Clin Endocrinol 18, 175-181.

Hanssen, T., Heyden. T., Sundberg. I., Alfredsson, G., Nybäck, H. and Wetterberg. L. (1980). Propranolol in schizophrenia. Clinical, melatoblic and pharmacological findings. Arch Gen Psychiatry 37, 685-690.

Hanssen. T. and Wetterberg, L. (1983). Serum levels of melatonin in schizophrenic patients. A possible indicator of beta-adrenergic function and of responsiveness to beta-adrenergic blocking drugs (propranolol). Neuroendocrinol Lett 5, 413.

Iguchi. H., Kato, K. and Ibayashi, H. (1982a). Age-dependent reduction in serum melatonin concentrations in healty human subjects. J Clin Endocrinol Metab 55, 27-29.

Iguchi, H., Kato, K.-I. and Ibayashi. H. (1982b). Melatonin serum levels and metabolic clearance rate in patients with liver cirrhosis. J Clin Endocrinol Metab 54, 1025-1027.

Klein, D.C. (1984). Melatonin and puberty. Letters. Science. 224, 6.

Lemaitre, B.J., Bouillie, J. and Hartmann, L. (1981). Variations of urinary melatonin excretion in humans during the first 30 years of life. Clin Chim Acta 110, 77-82.

Lenko. H.-L., Land. U., Aubert, M.L., Paunier, L. and Sizonenko, P.C. (1982). Hormonal changes in puberty, VII. Lack of variation of daytime plasma melatonin. Clin Endocrinol metab 54, 1056-1059.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., Markey, S.P. (1980). Light suppresses melatonin secretion in humans. Science 210, 1267-1269.

Lissoni, P., Mauri, R., Resentini, M., Morabito, F., Fraschini, F., Fumagalli, P. and Santagostino, A. (1984). Beta-endorphin levels after melatonin administration in man. J of steroid biochem 20, 1458.

Mendlewicz, J., Linkowski, P., Branchey, L., Weinberg, U., Weitzman, E.D. and Branchey, M. (1979). Abnormal 24 hour pattern of melatonin secretion in depression. Lancet ii, 1361.

Owen, F., Ferrier, I.N., Poulter, M. and Crow, T.J. (1983). Hydroxy-indole-O-methyltransferase activity in human pineals: A comparison of controls and schizophrenics. Clin Endocrinol 19, 313-317.

Penny, R. and Goebelsmann, U. (1984). Effect of estradiol on plasma melatonin levels. Endocrinol Invest 7, 55-57.

The Pineal Organ - Photobiology-Biochronometry-Endocrinology. Oksche, A., Pévet, O. (Eds.) (1981) Elsevier/North-Holland Biomedical Press, Amsterdam. New York. Oxford. 1-366.

The Pineal and its Hormones. Reiter, R.R. (Ed.) (1982). Progress in clinical and biological research. Alan R. Liss, Inc. New York, 1-295.

The Pineal Gland. Relkin, R. (Ed.) (1983). Elsevier Biomedical. New York. Amsterdam. Oxford, 1-311.

The Pineal Gland and Its Endocrine Role. Axelrod, J., Fraschini, F., Velo, G.P. (Eds.) (1983). Plenum Press, New York and London, Published in cooperation with NATO Scientific Affairs Division. 1-604.

Sizonenko, P.C., Lang, U. and Aubert, M.L. (1982). Neuro-endocrinologie de la puberté. Rôle de la mélatonine chez l'homme. Ann Endocrinol Paris 43, 453-464.

Smith, J.A., Mee, T.J.X., Padwick, D.J. and Spokes, E.G. (1981). Human post-mortem pineal enzyme activity. Clin Endocrinol 14, 75-81.

Touitou, Y., Fevre, M., Lagoguey, M,. Carayon, A., Bogdan, A., Reinberg, A., Beck, A., Cesselin, F., Touitou, C. (1981). Age- and mental health-related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man. J Endocrinol 91, 467-475.

Touitou, Y., Fèvre, M., Bogdan, A., Reinberg, A., De Prins, J., Beck, H. and Touitou, C. (1984). Patterns of plasma melatonin with ageing and mental condition: Stability of nyctohemeral rhythms and differences in seasonal variations. Acta Endocrinol 106, 145-151.

Vaughan, G.M. (1984). Melatonin in humans. Pineal Research Reviews 2, 141-201.

Verhoeven, W., Van Ree, J., Westenberg, H., Krul, J., Brouwer, G.J., Thijssen, J., de Wied, D., van Praag, H,, Ceulemans, D. and Kahn, R. (1984). Clinical, biochemical, and hormonal aspects of treatment with des-tyr^{1}-gamma-endorphin in schizophrenia. Psychiatry Research 11, 329-346.

Waldenlind, E., Ekbom K., Friberg, Y. Sääf, J. and Wetterberg L. (1984).Decreased nocturnal serum melatonin levels during active cluster headache periods. Opusc. Med. In press.

Waldhauser, F., Lynch, H.J. and Wurtman, R.J. (1984a). Melatonin in human body fluids: clinical significance. In The Pineal Gland, Reiter, R. (Ed.) Raven Press, New York, 345-370.

Waldhauser, F., Frisch, H., Waldhauser, M., Weiszenbacher, G., Zeitlhuber, U. and Wurtman, R.J. (1984b). Fall in nocturnal serum melatonin during prepuberty and pubescence. Lancet i, 362-365.

Werner, S., Brismar, K., Wetterberg, L. and Eneroth, P. (1981). Circadian rhythms of melatonin, prolactin, growth hormone and cortisol in patients with pituitary adenomas, empty sella syndrome and Cushing's syndrome due to adrenal tumours. In Melatonin: Current Status and Perspectives, Birau, N. and Schloot, W. (Eds.) Pergamon Press Ltd. Oxford, 357-363.

Wetterberg, L., Beck-Friis, J., Aperia, B. and Petterson, U. (1979). Melatonin/cortisol ratio in depression. Lancet ii, 1361.

Wetterberg, L., Aperia, B., Beck-Friis, J., Kjellman, B.F., Ljunggren, J.-G., Nilssone, Å., Petterson, U., Tham, A. and Undén, F. (1982). Melatonin and cortisol levels in psychiatric illness. Lancet ii, 100.

Wetterberg, L., Iselius, L. and Lindsten, J. (1983). Genetic regulation of melatonin excretion in urine. Clinical Genetics 24, 399-402.

Wetterberg, L., Beck-Friis, J., Kjellman, B.F. and Ljunggren, J.-G. (1984a). Circadian Rhythms in Melatonin and Cortisol Secretion in Depression. In Frontiers in Biochemical and Pharmacological Research in Depression, Usdin, E., Åsberg, M., Bertilsson, L. and Sjöqvist, F. (Eds.) Raven Press, New York, 197-205.

Wetterberg, L., Sääf, J., Norén, B., Waldenlind, E., Friberg, Y. (1984b). Interference with the radio-immunoassay of melatonin by dimethyl phtalate. J of steroid biochem 20, 1475.

Wirz-Justice, A. and Arendt, J. (1979). Diurnal, menstrual cycle and seasonal indole rhythms in man and their modification in affective disorders. In Biological Psychiatry Today, Obiols, J., Ballús, C., González Monclús, E. and Pujol, J. (Eds.) Elsevier/North-Holland, Biomedical Press, Amsterdam, 294-302.

Yuwiler, A. and Brammer, G. (1981). Neonatal hormone treatment and maturation in the pineal noradrenergic system: hydrocortisone and thyroxine. J Neurochem 37, 985-992.

The Pineal Gland
Current State of Pineal Research
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STRUCTURAL AND ULTRASTRUCTURAL ANALYSIS OF THE PINEAL OF PRIMATES; ITS INNERVATION

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Since 1932, when Rio Hortega gave his classic account of the state of knowledge at that time concerning the structure of the pineal gland, many advances have taken place in this subject. These have been in large part due to the introduction of electron-microscopy and the development of histochemistry. Of particular importance has been the growth of knowledge concerning its innervation.

Rio Hortega considered that nerve fibres reached the pineal from both the central nervous system and the peripheral nervous system. He stated that bundles of myelinated fibres derived from the commissura habenularum and the commissura posterior enter the organ and shortly afterwards lose their myelin sheaths and become dissociated among the parenchymal cells; thus, he left the question open as to whether they actually terminated in its substance. The fibres of peripheral origin he believed to come from the tela chorioidea and to form a nerve plexus within its substance; this was in accord with the view of Ramon y Cajal (1904, 1911) who regarded these fibres as being sympathetic and stemming from the ganglion cervicale superius. Ramon y Cajal referred to the denseness of this intrapineal nerve plexus and stated the pineal "is perhaps one of the richest and best supplied of all the glands".

Rio Hortega viewed the occurrence of intrapineal ganglionic nerve cells as rare. He did not mention the report given by Kolmer (1929) of the constant occurrence of numerous ganglion cells within the distal part of the pineal gland of monkeys and did not refer to the description in man and the monkey as well as the dog and the goat of the "nervus conarii" provided by Kolmer and Löwy (1922) who described this nerve as a well-defined bundle of nerve fibres which appears at the posterior pole of the pineal and courses in relation to the vena cerebri magna toward the tentorium cerebelli. Additional evidence of the occurrence of ganglion cells within the pineal of monkeys was given by Levin (1938) who reported more than 2,000 in the bonnet monkey (*Macaca radiata*).

Le Gros Clark (1940) undertook a more detailed study of the nervus conarii in man and the Rhesus monkey (*Macaca mulatta*) during which he commented on the resemblance the intrapineal nerve cells of the monkey bore to the ganglion cells of the autonomic nervous system. He described these intrapineal cells as being usually multipolar and traced

the nervus conarii from a central core of neuropil containing them into the wall of the sinus rectus in close relation to the branches of a choroidal artery. After section of the nervus petrosus major in macaque monkeys Kenny (1961) reported degeneration of nerve fibres within the nervus conarii and degeneration of the nerve cells within the intrapineal neuropil, which 30 days after operation was largely replaced by a cellular mass resembling glial tissue containing two cells with the appearance of necrotic neurons. Kenny interpreted these findings as indicating the presence of preganglionic parasympathetic nerve fibres within the nervus conarii and regarded the nerve cells of the neuropil as the cells of origin of postganglionic parasympathetic nerve fibres. Later David et al. (1975) indicated that acetycholinesterase can be demonstrated in these cells. In the same study these workers also found many non-myelinated nerve fibres and nerve terminals ending in relation to intrapineal blood-capillaries of the monkey degenerated after bilateral removal of the ganglion cervicale superius. This result indicated the existence of a postganglionic sympathetic contribution to the innervation of the gland and these workers concluded that it was mainly concerned with the blood-vascular component of the organ. Therefore, it would appear that the pineal gland of the monkey is innervated by both parasympathetic and sympathetic components of the autonomic nervous system.

Collections of nerve cells have been reported in the vicinity of the pineal gland of man by a number of investigators. Marburg (1909) found a small nerve ganglion above the organ in neonatal material and this ganglion was also noticed in an individual aged twenty years in a position between the suprapineal recess and the dorsal surface of the organ by Kenny (1965); however, Kenny reported its absence in older individuals. Pastori (1930) described a group of about 20 nerve cells at the posterior extremity of the organ associated with the nervus conarii. Møllgård and Møller (1973) verified the presence of these ganglia in the fetal gland and Møller (1978) reported another ganglion situated rostrally on the dorsal surface of the pineal closely related to the choroid plexus of the third brain ventricle. In this work Møller also described a tract of nerve fibres running from the habenular region which sometimes reached the ganglion of Pastori; this tract was associated with an intrapineal ganglion consisting of 15 to 30 nerve cells. However, as far as the occurrence of intrapineal nerve cells in adult humans is concerned the conclusion of Bargmann (1943), namely, they occur only occasionally, appears to be still valid.

In contrast, too, to the single nervus conarii of the monkey, in man the nervus conarii is present bilaterally (Kenny 1965). The two nerves run anteriorly beneath the floor of the vena cerebri magna from the dura mater of the tentorium cerebelli at the junction of this vein and the floor of the sinus rectus through a large arachnoidal formation, which was termed the "suprapineal arachnoid body" by Le Gros Clark (1940), towards the posterior pole of the pineal body. In its course, each nerve forms part of a neurovascular bundle but the fibres of the nerve, which penetrate the posterior pole at a number of sites, in many instances enter the organ independently of vessels. Within the pineal body the fibres can only be traced with certainty for a restricted distance, but, nevertheless, they can be seen to participate in the formation of a network extending through both the interlobular septa and

the parenchyma. In a study of human fetuses 60 to 150 days old Hülsemann (1971) showed that the principal innervation comes by way of this nerve, a finding which agrees with that of Kenny. Møllgård and Møller (1973) and Møller (1978) have also described in human fetuses a small unpaired midsagittal nerve, the "nervus pinealis", which courses through the subarachnoid space between the caudal portion of the pineal and the most rostral portion of the mid-brain in the general area of the subcommissural organ; however, this nerve has not been found in postnatal material and its role like that of the various ganglia that have been described in connection with the human pineal gland remains obscure.

The significance of the intrapineal fibres derived from the commissura habenularum and commissura posterior is also obscure. In human embryos Hochstetter (1921) found that fibres of commissural origin formed loops on the surface of the pineal that permitted them to return to the commissure on the opposite side of the mid-line. Kenny (1965) reported that in adult humans he observed a few commissural fibres intermingled with the adjacent substance of the pineal but was unable to trace any of these fibres deeply into it. However, Scharenberg and Liss (1965) succeeded in tracing individual nerve fibres derived from the commissures to the vicinity of pinealocytes with which they appeared to make contact. In the monkey (*Macaca mulatta*) Le Gros Clark (1940) concluded that the nerve fibres which entered the pineal substance from the commissures were aberrant fibres which returned to the commissures and were not concerned with the functional innervation of the organ. Nielsen and Møller (1975), on the other hand, in *Cercopithecus aethiops* found a tract of nerve fibres which ran from the posterior commissure deeply into the pineal before becoming lost. They were unable to determine whether these fibres were pinealopetal or pinealofugal but were able to find an occasional fibre within the pineal terminating as an end-bulb close to a pinealocyte. David et al. (1975) also found that in *Macaca mulatta* nerve fibres entered the pineal from the commissures and, in contrast to the earlier study of Le Gros Clark, concluded after placing lesions in the habenula that a significant number of fibres from the commissura habenularum terminated in the intrapineal neuropil of this primate. They, therefore, stated that the pineal of the monkey is innervated not only by the peripheral nervous system but by fibres of central origin stemming from the habenular region and indicated that the role of the fibres entering the organ from the posterior commissure remained unknown.

It is clear that our knowledge of the innervation of the primate pineal gland is by no means complete. It has only been intensively studied in two members of this large order, man and monkey, and with respect to the innervation of the pineal of the monkey, which is the only primate from which relevant experimental results have been obtained, there are many questions. What is the significance in functional terms of the neural component of the macaque which appears to have preganglionic parasympathetic nerve fibres and fibres derived from the habenular commissure ending within it? What is the significance of the intrapineal fibres derived from the posterior commissure? Are the endings of postganglionic sympathetic nerve fibres confined to intrapineal blood-vessels? What is the site of termination of the postganglionic parasympathetic nerve fibres which appear to exist? Are

there nerve endings directly related to the pinealocytes and, if they do exist, what is their origin? All of these questions remain to be answered.

REFERENCES

Bargmann W (1943): Die Epiphysis Cerebri. In Handbuch der Microskopischen Anatomie des Menschen. Hrsg. W.v. Möllendorff. Springer, Berlin. Bd. 6, T.4, pp. 309-502

David GFX, Umberkoman B, Kumar K, Anand Kumar TC (1975): Neuroendocrine signficance of the pineal. In Brain-endocrine Interaction II: The Ventricular System in Neuroendocrine Mechanisms. Ed. by KM Knigge, DE Scott, H Kobayashi, S Ishii. Karger, Basel. pp 365-375

Hochstetter F (1921): Über die Entwicklung der Zirbeldrüse des Menschen. Anat. Anz. 54(Erg. Bd.), 193-198

Hülsemann M (1971): Development of the innervation in the human pineal organ. Light and electron microscopic investigations. Z. Zellforsch. 115, 396-415

Kenny GCT (1961): The "nervus conarii" of the monkey (an experimental study). J. Neuropath. exp. neurol. 20, 563-570

Kenny GCT (1965): The innervation of the mammalian pineal body (a comparative study). Proc. Aust. Assoc. Neurol. 3, 133-140

Kolmer W (1929): Ganglienzellen als konstanter Bestandteil der Zirbel von Affen. Ztschr. ges. Neurol. u. Psychiat. 121, 423-428

Kolmer W, Löwy R (1922): Beiträge zur Physiologie der Zirbeldrüse. Pflüg. Arch. ges. Physiol. 196, 1-14

Le Gros Clark WE, (1940): The nervous and vascular relations of the pineal gland. J. Anat. 74, 471-492

Levin PM (1938): A nervous structure in the pineal body of the monkey. J. comp. Neurol. 68, 405-409

Marburg O (1909): Zur Kenntnis der normalen und pathologischen Histologie der Zirbeldrüse. Die Adipositas cerebralis. Arb. Neurol. Inst. Wien. Univ. 17, 217-279

Møller M (1978): Presence of a pineal nerve (nervus pinealis) in the human fetus: a light and electron microscopical study of the innervation of the pineal gland. Brain Res. 154, 1-12

Møllgård K, Møller M (1973): On the innervation of the human fetal pineal gland. Brain Res. 52, 428-432

Nielsen JT, Møller M (1975): Nervous connections between the brain and the pineal gland in the cat (Felis catus) and the monkey (Cercopithecus aethiops). Cell Tiss. Res. 161, 293-301

Pastori G (1930): Ein bis jetzt noch nicht beschriebenes sympathisches Ganglion zum Nervus conari [sic] sowie zur Vena magna Galeni. Z. ges. Neurol. Psychiat. 123, 81-90

Ramon y Cajal S (1904): Textura del Sistema nervioso del hombre y de los vertebrados. Moya, Madrid. t.2, p.758

Ramon y Cajal S (1911): Histologie du système nerveux de l'homme et des vertébrés. Ed. ... rev. ... tr. ... par L. Azoulay. Maloine, Paris. t.2, p.486

Rio Hortega P del (1932): Pineal Gland. In Cytology and Cellular Pathology of the Nervous System. Ed. by W Penfield. Hoeber, New York. Vol.2, pp. 637-703

Scharenberg K, Liss L (1965): The histologic structure of the human pineal body. Progr. Brain Res. 10, 193-217

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OBSERVATIONS CONCERNING THE PINEAL MORPHOLOGY OF PRIMATES

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The extant primates represent the endforms of at least four phylogenetic stems: the Old World Monkeys and Apes, the New World Monkeys, the Prosimiae and the Tarsiiformes. The Old World Monkeys and Apes (Catarrhini) and the New World Monkeys (Platyrrhini) are probably related at the base of their radiation by a common link, which in turn probably originated from a remote and primitive tarsiiform population which became extinct. The modern Prosimiae and the modern Tarsius are not phylogenetically related to other extant primates; in the phylogenetic sense they are typical endforms. Precision concerning the species to be considered is necessary as they could be separated from each other by a span of time almost as long as the tertiary period.

The place of the tupaiids is still uncertain. It may be that they are very primitive prosimians or they may belong to an order of their own in which case the question arises whether they have any phylogenetic relation to the primates. The modern tupaiids are the most primitive eutherian representatives and share many anatomical features with the most primitive extant marsupial Didelphys which exhibits the most primitive form of mammalian pineal organ, that is, a hollow, hemispherical structure whose cavity (recessus pinealis) opens into the third ventricle of the brain (Hofer et al., 1976). Although the matter remains controversial, in this study Tupaia glis is considered to be a survivor of a group of eutherians ancestral to the primates and probably to other eutherian groups as well. Because of this, the pineal region of the brain of T. glis has been investigated in this work together with that of the Old World monkey Macaca fascicularis, the New World monkeys Callithrix callitricha, Cebus apella and Saimiri sciureus, and the prosimians Galago crassicandatus, Nycticebus coucang and Lemur (species unknown, ? fulvus).

Little is known about the primate pineal gland (body) (Suzuki, 1938; see reviews by Bargmann, 1943, and Vollrath, 1981). It is typically a distinct, solid structure situated between the commissura habenularum and the commissura caudalis (posterior) which is covered on its ventricular surface by the subcommissural organ (SCO); in form, it is mostly knob-, cone- or spindle-shaped. It is more or less tilted over the tectum mesencephali, occasionally coming into very close contact with the mesencephalon, and its dorsal aspect is covered by the recessus suprapinealis of the caudal part of the plexus choroideus of the third ventricle. Its histological and cytological features appear to be essentially the same as those observed in other mammalian pineal glands. Accordingly, this study is confined to selected points of primate pineal anatomy.

Material and Methods

The animals used in this study were obtained from a dealer. For light-microscopic studies the animals were anaesthetized (ether, nembutal) and the blood was washed out with normosol, hemacel or periston N. Subsequently they were perfused with the fixative (Bouin's fluid, formalin) from the left ventricle or from the aorta. The whole brains were embedded in paraffin and sectioned at either 10 µm or 12 µm. The following dyes were used to stain the tissues: Nissl, azan, chromalum-hematoxylin-phloxin, iron hematoxylin. Silver methods after Palmgren, Bodian and Perdrau were used to impregnate nervous tissue.

For electron-microscopic studies the animals were initially treated as for the light-microscopic studies; then they were perfused with a solution of 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for approximately 30 to 60 minutes. The brains were dissected and the pineals were put in the same fixative for 6 to 12 hours; subsequently the tissues were washed several times in 0.1M cacodylate buffer and postfixed in a solution of 2% osmium tetroxide in 0.1M cacodylate buffer; the tissues were dehydrated in a graded series of acetone and stained for 60 minutes "en bloc" in a solution of 1% uranyl acetate and 5% phosphotungstic acid. Vestopal W was used for embedding; the tissues were subsequently sectioned with glass knives on a Reichert ultramicrotome and studied with the aid of a Zeiss EM 9S electron microscope at 60kv.

The following primate species were available for electron-microscopic studies: Callithrix callitricha, Saimiri sciureus, Cebus apella and Macaca fascicularis.

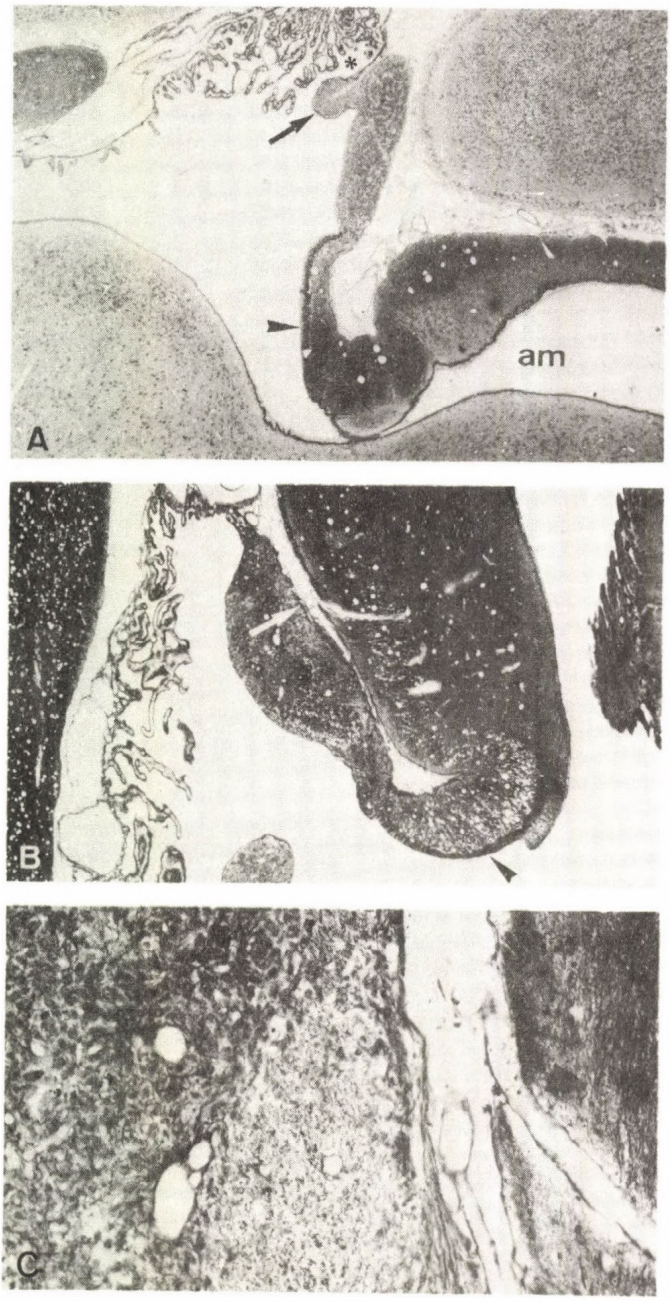


Fig.1.
Sagittal sections, pineal area
A) **Tupaia glis**, ♀, pregnant, 10 µm, chromalum-hematoxylin-phloxin (Gomori). arrow: comm. hab.; arrowhead: SCO and comm. posterior; asterisk: recessus suprapinealis; am: aquaeductus mesencephali.
B) **Lemur** sp., 10 µm (Gomori). The ventricular surface of the pineal entirely immersed in the CSF. Arrow: comm. hab. (situated in the parenchyma of the pineal body); arrowhead: SCO and comm. posterior.
C) **Lemur** sp., 10 µm, obj. 16x, silver impregnation after Palmgren. Comm. hab. encompassed by pineal parenchyma.

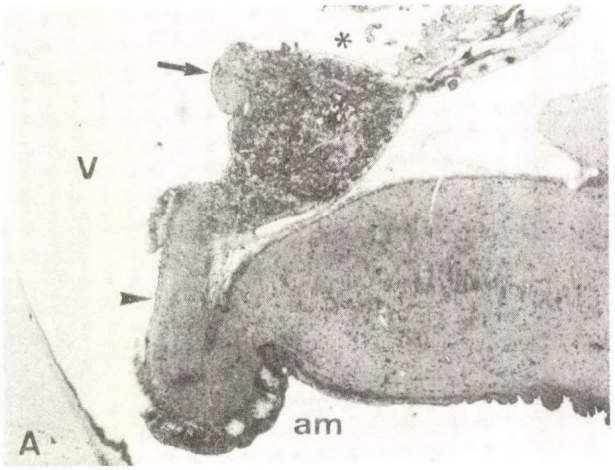
Observations

In Tupaia glis the pineal gland (Fig. 1a; see Hwang, 1982) is a parenchymatous bar, part of which projects over the commissura habenularum; the dorsal leg of the U-shaped commissure is continuous with the ventral wall of the recessus suprapinealis which is related inferiorly to the dorsal surface of the pineal body. The space between the two legs of the commissura habenularum contains extremely loose arachnoidal tissue and a few blood vessels. In Tupaia the bundles of the commissura habenularum and of the commissura caudalis are continuous with the parenchyma of the organ.

In Lemur sp. the organ is distinctly different from that of Tupaia in the two following features (Fig. 1b,c): first, its distal end is directly connected to the plexus choroideus; secondly, the commissura habenularum is situated within its parenchyma and has no relation to any part of the plexus choroideus. Indeed, the anatomical arrangement of the pineal region in this animal is highly specialized and divergent from the more primitive condition found in Tupaia in the following respects: (1) the pineal organ is not situated between the two commissures since the pineal parenchyma surrounds the commissura habenularum, (2) the nerve bundles of the cross-sectioned commissura habenularum are not arranged as a circumscribed tract but are dispersed in the pineal parenchyma, (3) there is no relation between the commissura habenularum and the plexus choroideus, (4) the recessus pinealis is absent, the ventricular surface of the pineal bulging into the third ventricle, and (5) a typical recessus suprapinealis is also missing.

In the lorisiform prosimian Nycticebus coucang the anatomy of the pineal complex (Fig. 2) can be readily derived from the condition found in Tupaia. The pineal body is typically situated, that is, between the commissures. The recessus suprapinealis is not remarkable; the portion of the pineal complex delimiting the recessus pinealis forms a slight convexity bathed by the cerebrospinal fluid (CSF) of the third ventricle. The commissura habenularum is incorporated with the pineal body and in sagittal sections it appears as a small dorsally situated knob. The dorsal surface of the pineal body is totally covered by the ventral lamina of the recessus suprapinealis. The pineal complex of the other lorisiform prosimian available for study, namely, Galago crassicaudatus is macroscopically similar to that of Nycticebus. In sagittal sections, the pineal body in Nycticebus appears more conical and less spherical in shape than in Galago and in contrast to Galago the dorsal surface of the commissura habenularum is covered with pineal parenchyma; these differences may be within the range of normal variation.

In the New World monkey Saimiri sciureus (Fig. 3) the pineal complex has the same general configuration as that found in Nycticebus coucang and Galago crassicaudatus. In the



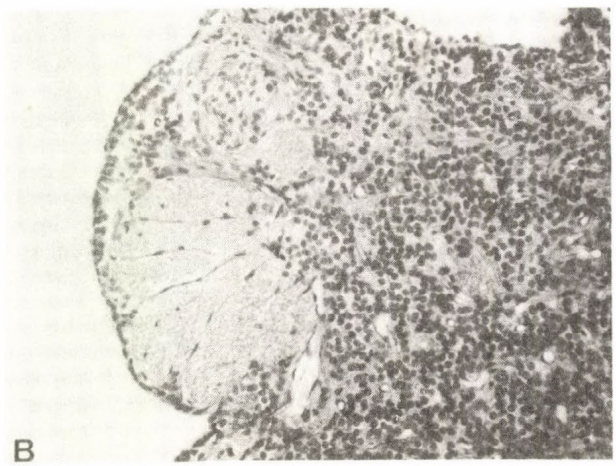


Fig.2.
Nycticebus coucang, sagittal section of the pineal area; 10 µm, (Azan).
A) Survey; V: third ventricle; arrow: comm. habenularum; arrowhead: comm. caudalis with parts of the SCO; asterisk: recessus suprapinealis; am: aquaeductus mesencephali.
B) Comm. habenularum partly covered by pineal parenchyma; x 16.

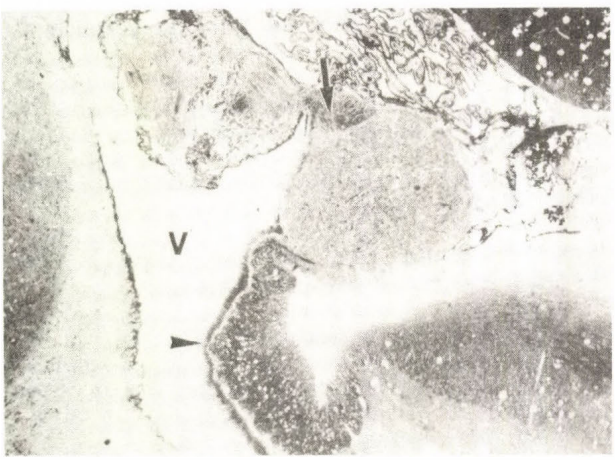


Fig.3.
Saimiri sciureus, adult ♀; sagittal section of the pineal area; 10µm; iron hematoxylin (Heidenhain). Note the globular shape of the pineal body. V:third ventricle; arrow: comm. hab.; arrowhead: SCO and comm. caudalis.

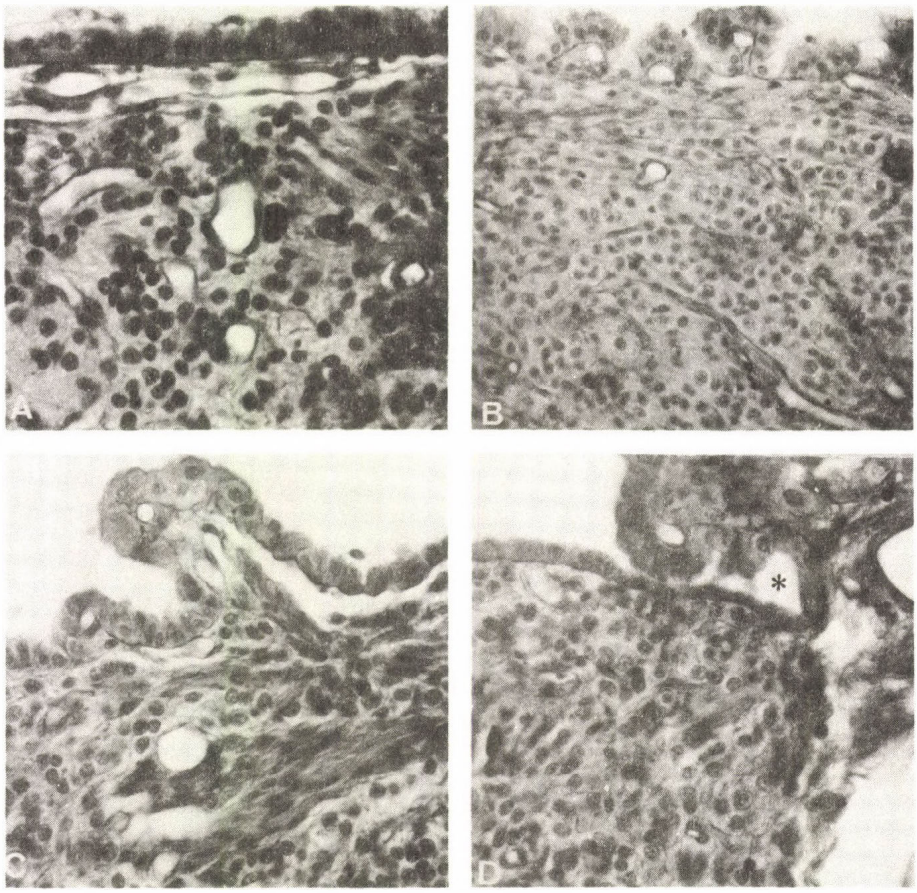


Fig.4.
Ventral lamina of the recessus suprapinealis; the coalescence is in all of these cases complete. Note vascularization of the pineal parenchyma underneath of the epithelial layer.

A) **Galago crassicaudatus**, cross-section, 10 µm, obj. 40x (Gomori). The epithelium is simple cuboidal exhibiting kinocilia.

B) **Macaca fascicularis**, adult, sagittal, 10 µm, obj. 25x (Gomori). Simple folds of the lamina containing capillary loops.

C) **Galago crassicaudatus**, adult, sagittal, 10 µm, obj. 40x (Gomori). Two epithelial folds of different size containing blood vessels.

D) **Galago crassicaudatus**, same animal as in C; technical data as in C. The extremity (asterisk) of the recessus suprapinealis; the cuboidal epithelium, which is coalescent with the pineal body, is continuous with the epithelium of the plexus; note differences in the two kinds of cells, which are of the same origin.

Fig.5.
Saimiri sciureus,
sex unknown. Layer of cells of the lamina of the suprapineal recess coalescent with the pineal body. The epithelial cells of the recess form a fold containing blood vessels (asterisks); note the abundant microvilli (arrowheads). The epithelial cells are upholstered by loose arachnoidal tissue; note the collagen fibrils (arrows). There is no organized arachnoid tissue separating the elements of the recessus from the dorsal surface of the pineal body. The pineal cells are dorsally covered by a dense texture of filamentous processes of astrocytes.
x 2850.

Old World monkey Macaca fascicularis the pineal gland is characterized by the presence of a dorsal and a ventral lamella connecting the organ dorsally with the commissura habenularum and ventrally with the commissura caudalis; glial tissue lacking parenchyma and nerve fibres assists in this connection. The ependyma covering the ventral part of the organ in Macaca fascicularis is directly continuous with the ependyma of the SCO. In adult specimens it is distinctly different from the elongated secretory ependyma of the SCO. In newborn individuals it is difficult to discriminate between the two because of the presence in this situation of fetal ependyma which is also composed of elongated cells; however, no evidence of secretory activity in the ependyma covering the ventral part of the organ is present.

The recessus suprapinealis of the investigated species of primates provides a number of interesting observations. The epithelial cells of the plexus choroideus lining the ventral lamina of the recessus suprapinealis adjacent to the dorsal surface of the pineal show different features from those of the typical choroidal epithelial cells in the same individual (Fig. 4); they are flatter and their brush-border is less distinctly developed. Additionally, after the use of routine stains, e.g., azan and chromalum-hematoxylin-phloxin, these modified cells stain slightly, but, nevertheless, distinctly differently from the other choroidal epithelial cells being paler in appearance.

In cross-sections the recessus suprapinealis shows a median area in contact with the dorsal surface of the pineal body while the lateral parts are free. The choroidal epithelial cells of these free portions are typical in type and the leptomeningeal tissue adjacent to these cells contains numerous collagen fibrils forming part of a conspicuous layer of connective tissue which helps to separate the recess from the extracerebral CSF. In the median area the ventral lamina of the recess coalesces with the pineal. The coalescence is characterized by alterations in the appearance of the structures involved.

In Saimiri sciureus the area of coalescence (Fig. 5) shows only a single layer of loosely textured leptomeningeal tissue, with collagen fibrils running in various directions, separating the modified choroidal epithelium from a densely textured layer of filamentous processes belonging to astrocytes also running in many different directions. This astrocytic layer, which corresponds to the lamina gliae superficialis of the brain, encompasses the pineal parenchyma to which it is attached without interruption by any intervening space. In Cebus apella (Fig. 6) the leptomeningeal connective tissue layer is absent and the layer of modified choroidal epithelial cells rests directly upon the lamina gliae superficialis in which the filamentous processes of astrocytes are especially numerous. Fibrillary astrocytic processes occur throughout the pineal of this species, a

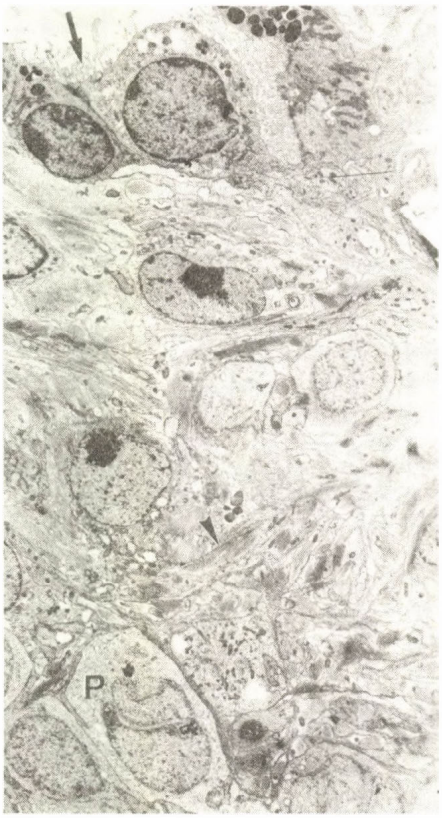
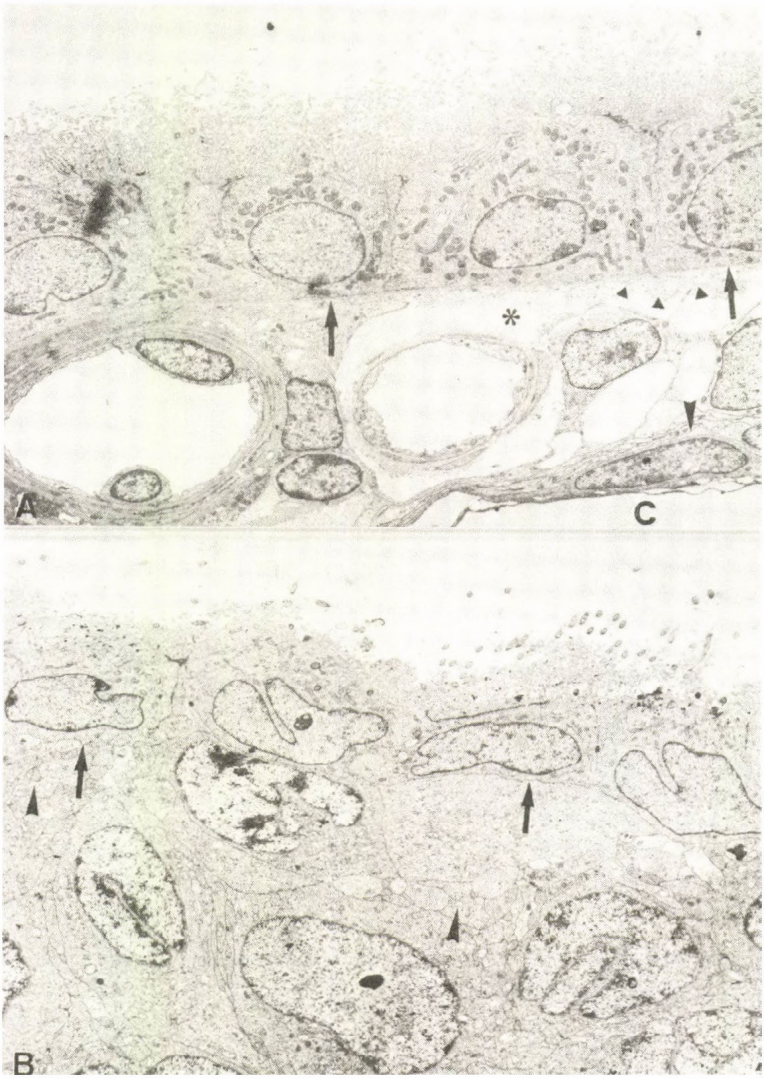


Fig.6.
Cebus apella;
the layer of cells of the suprapineal recess is coalescent with the pineal body. The microvilli (arrow) of the cells of the ventral lamina are less numerous than in regular plexus cells. There is no arachnoid layer; collagen bundles are entirely absent. The section meets the lamina of the recessus suprapinealis somewhat obliquely; therefore cells of the ventral lamina of the recess are situated within the thick layer of a dense texture of astrocytic processes (arrowhead); P: pineal cell. x2850.

Fig.7.
Topographical relation of the recessus suprapinealis to the dorsal surface of the pineal body in two different stages.

A) **Macaca fascicularis**; lateral parts of the recessus suprapinealis which is still free of the dorsal surface of the pineal body. The cells of the ventral lamina of the recessus are typically developed plexus cells; note the basement membrane (arrows). The tissue ventral to the latter is leptomeninx containing blood vessels; there is a wide perivascular space (asterisk) encompassing one of the vessels. Note the distinct layer of connective tissue (arrowhead) separating the wall of the suprapineal recess from the cisterna (C); note the collagen bundles (small arrowheads); x 5700.



B) **Callithrix callithricha**; most complete coalescence of the ventral epithelial lamina of the recessus suprapinealis with the pineal body. The simple layer of flat epithelial cells exhibits a distinct basal lamina (arrows); the number of microvilli is distinctly less than in the same cells of other species. Note the deep indentations of the nuclear envelopes. Filamentous processes of astrocytes appear in clusters or in isolation (arrowheads); they never form a continuous layer; x 5700.

feature which appears to be a general characteristic of the New World monkeys (for references see Vollrath, 1981; Wartenberg, Saimiri, 1968; cf. Hülsemann, Macaca, 1967). The modified choroidal epithelial cells of the median portion of the ventral lamina of the recess in Cebus possess fewer microvilli than the epithelial cells of the free lateral portions of the ventral lamina of the recess in Macaca fascicularis (Fig. 7a). In Callithrix callitricha (Fig. 7b) the modified choroidal epithelial cells of the ventral lamina possess fewer microvilli than those of the other species; however, the deep infolding of their nuclear envelopes indicates a high cellular activity. In this species, the lamina gliae superficialis is absent and the modified choroidal epithelial cells are attached directly to the pineal parenchyma, a basement membrane being present; filamentous processes of astrocytes in relation to the recess occur only sporadically or in circumscribed clusters.

Conclusions

1. Alterations in the morphology of the mammalian pineal body are marked. However, the most primitive form represented in Didelphys has not been found to occur in any of the species examined in this study. Despite the fact that the pineal morphology of many prosimians is still unknown, it is very probable that the shape of the prosimian pineal and that of the New and Old World Monkeys and the Apes is derived from an ancestral type very similar to that of Tupaia. While this investigation indicates the pineal of Lemur sp. is different from that of other primates in several respects, no satisfactory explanation can be offered to account for this finding as only one specimen was available for study and the shape of the pineal in other Lemuridae is unknown.

2. In some species of the primates examined in this study part of the pineal parenchyma is atypically situated occurring between the bundles of the commissura habenularum or at least partly covering it. The signficance of this arrangement cannot be established due to the limited material available and, therefore, no statement can be made as to whether or not this area of parenchyma has the same function as the remaining parenchyma; in this connection a detailed study of its innervation is required.

3. In the primates studied, the ventral lamina of the recessus suprapinealis may coalesce with the dorsal surface of the pineal body in order to provide for the intracellular transport of hormones from the intrinsic cells of the pineal into the CSF. Although different layers of tissue can be found between the modified choroidal epithelium of the ventral lamina of the recessus suprapinealis and the pineal parenchyma, numerous blood capillaries are constantly situated beneath this epithelial layer; not infrequently the epithelium forms folds containing capillary loops. This arrangement

increases the surface area of the epithelium and, therefore, may facilitate the flow of functionally important substances from the intrinsic cells of the pineal through this area of epithelium into the CSF. This concept is speculative, however, and requires experimental proof; in particular, the direction of the blood flow in this area requires study. It need not be that movement of substances from the pineal into the CSF requires direct contact between the pineal parenchyma and the CSF. The recessus suprapinealis is homologous with the saccus dorsalis of reptiles; it is unlikely that a structure with such a long phylogenetic history would be without a significant function.

REFERENCES

Bargmann W (1943): Die Epiphysis Cerebri. In Handbuch der Microskopischen Anatomie des Menschen. Hrsg. W.v. Möllendorff. Springer, Berlin. Bd. 6, T.4, pp. 309-502

Hofer H, Oksche A, Merker G (1976): Atypische Formen des Pinealorgans des Säugetiere. Verh. Anat. Ges. 70, 97-102

Hülsemann M (1967): Vergleichende histologische Untersuchengen über das Vorkommen von Gliafasern in der Epiphysis cerebri von Säugetieren. Acta Anat. (Basel) 66, 249-278

Hwang BH (1982): Fluorescence and electronmicroscopic study of the tree shrew pineal organ. J. Neural Transm. 53, 193-212

Suzuki Y (1938): Beiträge zur Anatomie des Epithalamus, besonders der Epiphyse bei den Primaten. Arb. Anat. Inst. Sendai 21, 45-141

Vollrath L (1981): The Pineal Organ. In Handbuch der Microskopischen Anatomie des Menschen. Hrsg. A. Oksche und L. Vollrath. Springer, Berlin. Bd. 6, T.7, pp. 1-665

Wartenberg H (1968): The mammalian pineal organ: electron microscopic studies on the fine structure of pinealocytes, glial cells and on the perivascular compartment. Z. Zellforsch. 86, 74-97

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